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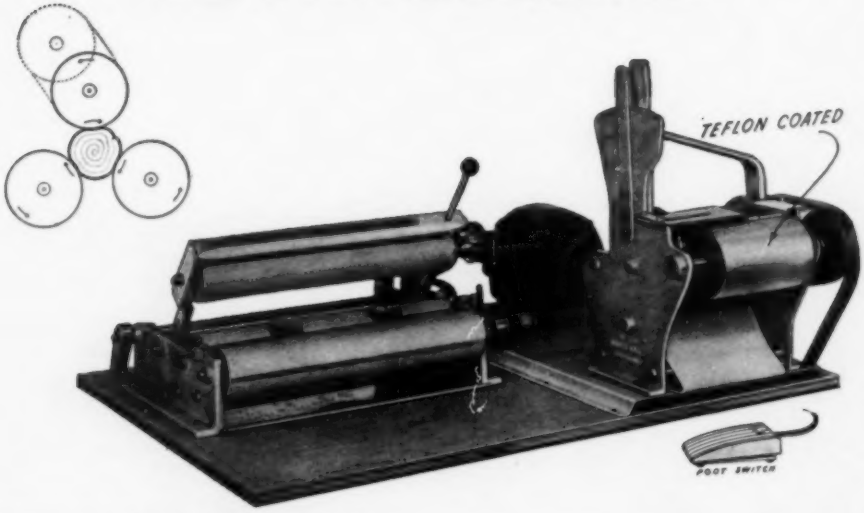

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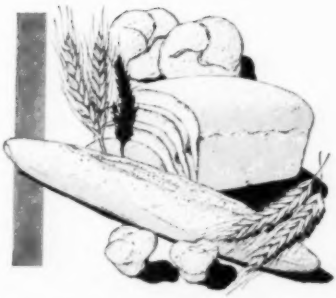

A Brief History of Milling and Baking by SCIENCE WRITER

CHAPTER IX. *Milling in the Early American Colonies*

EARLY AMERICAN GRAIN CULTURE

When the earliest settlers came to what is now the United States they found a dense wilderness sparsely occupied by Indians who depended on fishing and a nomadic agriculture practiced in small openings in the forest.

Corn, or maize, was the staple grain of the Indians, and of the early settlers. It was as important to them as wheat, barley or rye were to the Europeans. Wheat was soon imported and grown by the colonists.

The Indians had a practice totally different from European grain culture. They cultivated a small area around each corn hill. In the Old World it was the custom to scatter the wheat berry, barley and rye on the fields and let the grain fight it out with the weeds. The Indian cultivation was done with crude tools.

Seeing the advantages to be gained by this method the earliest New England colonists imported work animals from the home country and put them to the job of plowing and tillage. Apparently the results were satisfactory because crops of wheat and corn increased abundantly.

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With the increase in grain supplies it was necessary to found grist mills. These were probably post-mills powered by the wind, a type easier to build than the watermill. The latter soon took over, however, as the terrain of the Eastern United States is especially suitable.

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Wheat became a most important crop in New York, Pennsylvania, Maryland and on into the Middle West. New York City, Philadelphia, Baltimore, Richmond and Wilmington became major milling centers. Milling and traffic in grain played important parts in the phenomenal growth of New York and Philadelphia, as we shall see in our next chapter.

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The next chapter titled: "When New York and Philadelphia Were Milling Capitals" will be published soon.

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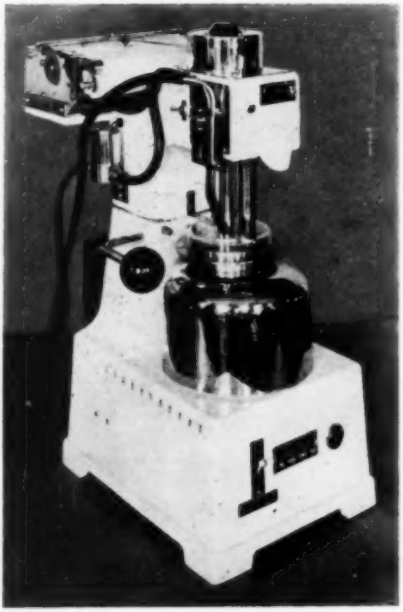

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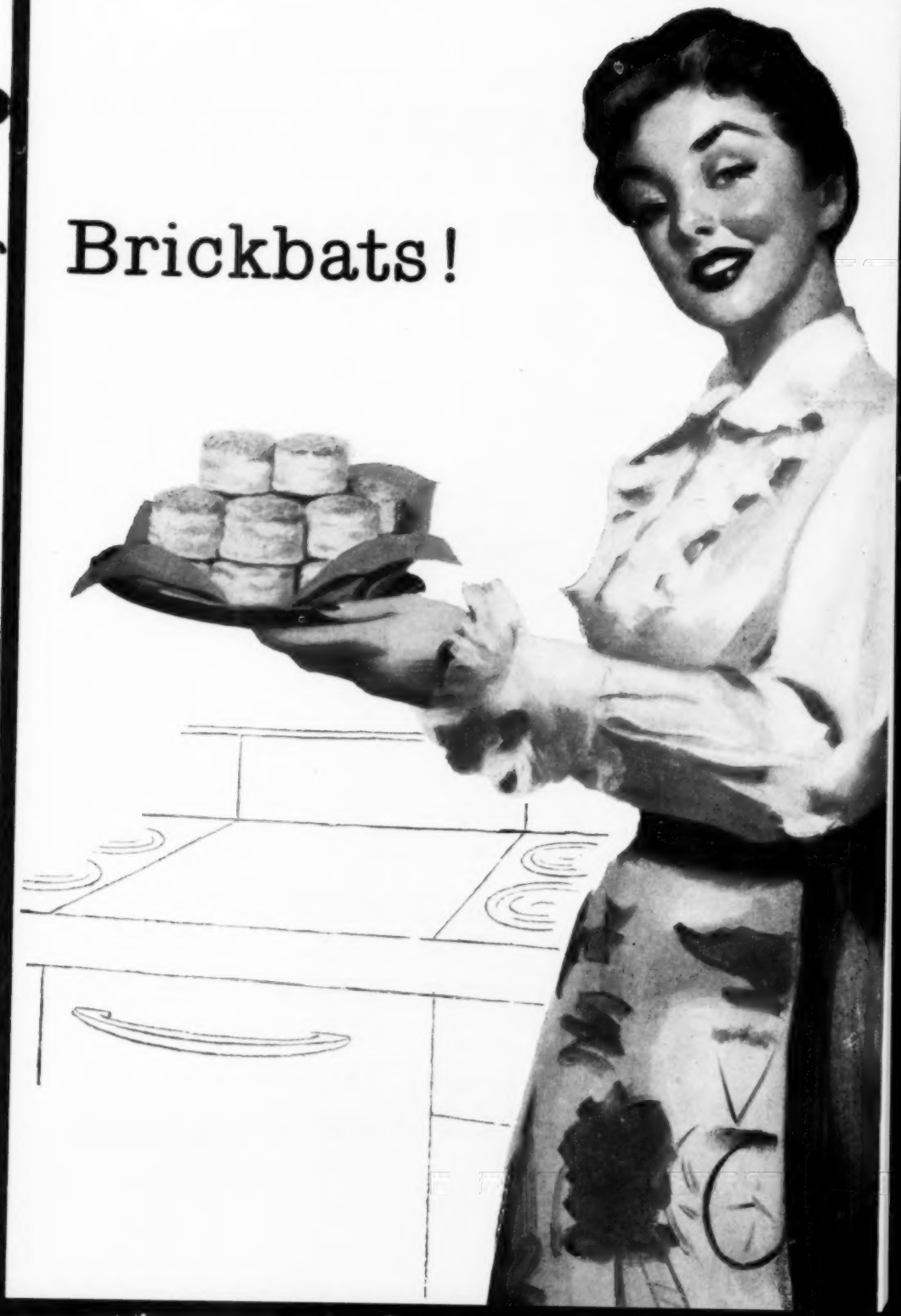

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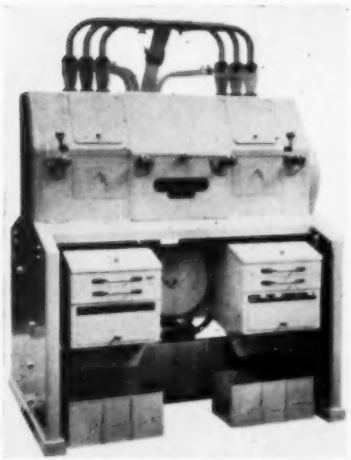

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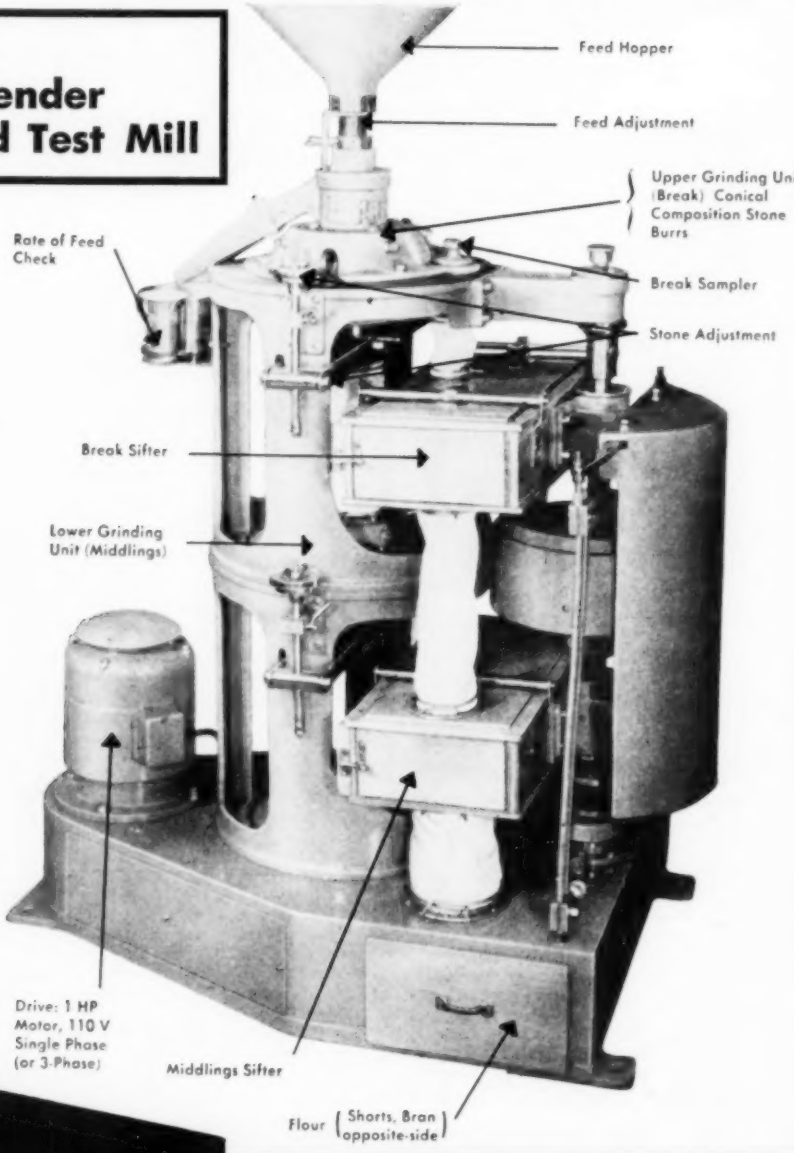

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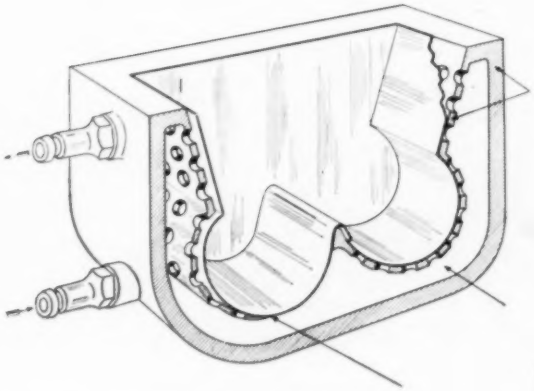

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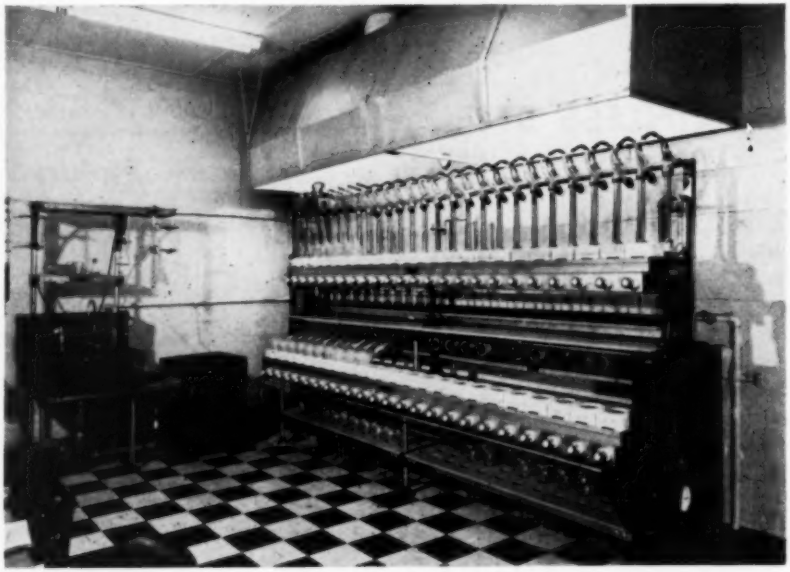

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CEREAL CHEMISTRY

VOL. 33 JULY, 1956 NO. 4

COMPARATIVE STUDY OF THE IMPROVING ACTION OF BROMATE AND IODATE BY BAKING DATA, RHEOLOGICAL MEASUREMENTS, AND CHEMICAL ANALYSES[1]

C. J. DEMPSTER, D. K. CUNNINGHAM, M. H. FISHER, I. HLYNKA, AND J. A. ANDERSON

ABSTRACT

The effect of the addition of potassium bromate and potassium iodate to dough from four widely different flours has been compared by the baking test, by structural relaxation experiments with the extensograph, and by chemical determinations of the disappearance of these improvers from dough.

Optimum loaf properties produced by the use of iodate occurred at lower equivalent concentrations than did these optima when bromate was the improver used. In addition, the magnitude of the improvement produced by iodate was less than that produced by bromate. While the reaction of bromate in dough is quite slow, the reaction of iodate is extremely rapid. Relaxation constants for iodate-treated doughs were larger than those for the corresponding control doughs, but both decreased at similar rates with increasing time. Relaxation constants for bromated doughs for all but one flour decreased with time at first but more slowly than those for controls. This was followed by an increase in the relaxation constant. Chemical analyses showed that, within the shortest time that a determination could be made, unleavened doughs consumed amounts of iodate greatly in excess of the optimum concentrations indicated by the baking tests. By contrast, the concentration of bromate in unleavened doughs decreased linearly with time at a rate dependent upon flour grade, although small amounts of bromate were rapidly consumed by two of the flours.

Results of this study suggest that the improving effect of various reagents may not arise from a single specific chemical reaction. Rather each improver may cause a different reaction, each of which produces changes in the rheological behavior of dough. Improvement in loaf properties results from the attainment of a particular, but not necessarily specific, combination of dough properties produced at a particular time in the time scale of the baking process by combinations of various reactions.

A promising line of attack in studying the chemistry of flour dough in recent years has been the application of rheological techniques to the study of changes in dough behavior produced by the addition of improving reagents. These studies have provided detailed quantitative

[1] Manuscript received January 11, 1956. Paper No. 154 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 334 of the Associate Committee on Grain Research (Canada).

descriptions of the behavior of doughs treated with improvers. A general postulate of a three-dimensional cross-linked or network structure for dough in which improvers act as cross-linking agents has been proposed to account for this rheological behavior.

Any adequate hypothesis of dough structure and improver reaction must account not only for the rheological behavior of dough but also for its baking behavior and for such changes as can be detected in it by chemical analysis. Accordingly, baking, rheological, and chemical tests have now been used in a comparative study of the effects of two improvers widely used in commercial baking, namely, potassium bromate and potassium iodate.

A number of investigators have reported on differences between the effects of bromate and of iodate. Sullivan, Howe, Schmalz, and Astleford (14) noted that iodate shows its effect more quickly than bromate and that the iodate effect is evident in dough taken from the mixer. Jorgensen (9), Sullivan *et al.* (14), and Holme and Spencer (8) presented data to show that improvers in general did not produce equivalent quantitative response in the baking test, and that less iodate than bromate was required to produce the same effect. Rheological experiments on the effect of bromate came with the development of the Brabender Extensograph (11, 12). The effect of iodate on extensograms was noted by Merritt and Bailey (10) and by Smith and Andrews (13). In the present study the technique of structural relaxation developed in this laboratory (2, 3) has been used to make a quantitative rheological comparison of the effect of bromate and iodate in dough.

The plan of investigation may be outlined as follows. Four widely different flours were subjected to the baking test to determine their response to the addition of increments of potassium bromate or potassium iodate. The particular concentration of bromate or iodate which produced optimum loaf properties for each flour was noted. Subsequently, the structural relaxation behavior of unleavened dough made from each of the four flours was examined. On the one hand, the doughs were untreated; on the other hand, the doughs were mixed to contain the optimum concentrations of either bromate or iodate. The third aspect of this investigation was a chemical determination of the disappearance of bromate or iodate from dough from each flour to which the optimum level of improver had been added initially.

This investigation lends itself to presentation in three separate sections, one for each phase of the study. Each section describes methods used and the results obtained, and includes a brief discussion. A final section is a general discussion of the entire investigation.

Materials

Flour. Four different grades of flour were employed in this study: a first patent from a mill mix of 13.6% protein; a flour from which the high-grade middlings and the clears have been removed, designated in this paper as intermediate; a clear; and a first germ flour from a mill mix of 12.6% protein. These flours were taken from appropriate streams in a commercial mill and had received no bleaching or improver treatment. Other particulars are listed in Table I.

TABLE I

SPECIFICATIONS OF FOUR FLOURS USED IN THIS STUDY

FLOUR STREAM	PROTEIN CONTENT	ASH CONTENT	FLOUR ABSORPTION
	%	%	%
First patent	12.4	0.40	62.1
Intermediate	13.0	0.56	61.7
Clear	15.2	0.92	62.9
First germ	12.7	0.96	58.1

I. Baking Experiments: Methods

The four flours were baked with increments of potassium bromate or potassium iodate to determine the response of these flours to improver addition. The basic A.A.C.C. malt-phosphate baking test was employed. However, it was desired that all four flours exhibit a positive response to the addition of both bromate and iodate. For this reason a yeast concentration of 2% was employed throughout the baking tests.

The response to bromate addition was determined for each flour in experiments where potassium bromate was added to dough in increments of 10 p.p.m. within the range 0 to 50 p.p.m. (flour basis). In parallel experiments, the response to iodate addition was determined by increasing its concentration in increments of 3 p.p.m. The range of concentrations used was 0 to 21 p.p.m. for both the patent and intermediate flours, 0 to 30 p.p.m. for the germ flour, and 0 to 36 p.p.m. for the clear flour.

Baking Experiments: Results and Discussion

The effect of increasing the concentration of bromate or iodate on the loaf properties of the bread made from each of the four grades of flour is summarized in Fig. 1, where loaf volume is plotted against the

concentration of the added improver. To facilitate a comparison of the effects of the two improvers, the improver concentration is expressed in terms of micro-equivalents (molecular weight/6 × 10^{-6}) per g. rather than on the usual parts-per-million basis.

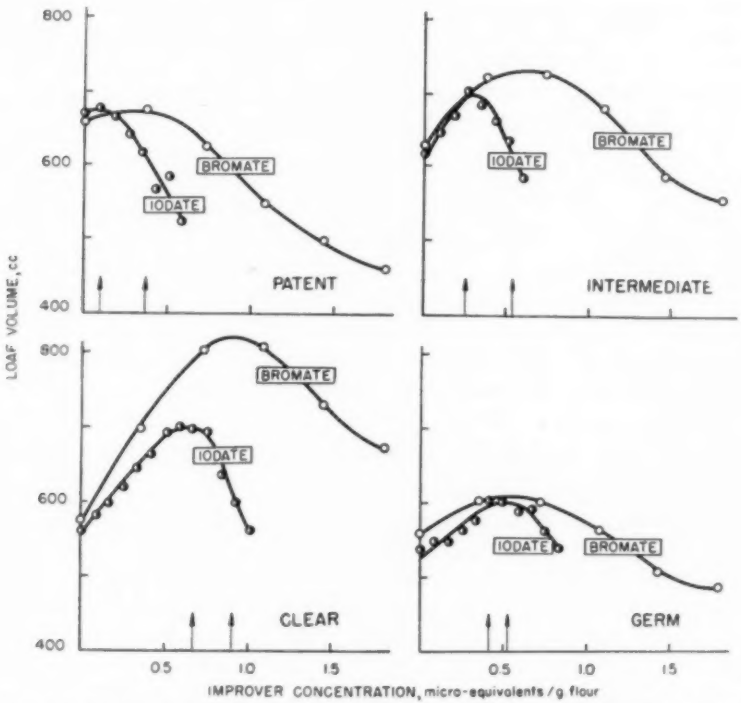

Fig. 1. Loaf volume response of four flours to increments of potassium bromate and potassium iodate.

Figure 1 shows that each of the four flours, when baked with 2% yeast, showed an improvement in loaf volume for additions of either bromate or iodate. This initial improvement was followed by a rather marked decline in loaf volume. However, the response of a given flour to the addition of bromate was quite different from its response to iodate in agreement with previous observations (8, 9). In general, potassium bromate appeared to produce a more marked improvement than did potassium iodate, but once the maximum had been attained iodate produced the more rapid deterioration in loaf properties. The arrows in Fig. 1 indicate the occurrence of optimum loaf properties as estimated by the baker on the basis of loaf volume and age character-

istics of the crust; these maxima were not produced by the addition of equivalent concentrations of bromate and iodate.[2] Optimum loaf volume for doughs containing potassium iodate occurred at a lower concentration than did the optimum for doughs containing bromate.

Figure 1 also shows that the improvement produced by either bromate or iodate varied considerably from flour to flour. Improvement was greatest for the clear flour, followed in order of decreasing response by the intermediate, germ, and patent flours. It will be noted that this placing corresponds to the decreasing protein contents of the flours.

II. Structural Relaxation Experiments: Methods

An experimental technique for using the Brabender Extensograph to determine the structural relaxation behavior of unleavened doughs has been described previously (2, 3) and may be summarized briefly as follows. Doughs were mixed in a G.R.L. mixer (6) operating at a speed of 36 r.p.m. at the drive plate of the mixer. Doughs were mixed in an atmosphere of nitrogen and contained 1% salt (flour basis). Potassium bromate or potassium iodate in solution was added as required to produce concentrations in the dough from each flour equal to that found in the baking experiments to be the optimum improver dosage. Some additional experiments were carried out with potassium bromate at levels of 15 and 30 p.p.m.

After mixing, doughs were allowed reaction times of 5 minutes, 1, 2, 3, and 4 hours. At the end of a desired reaction time, six test pieces were shaped on the extensograph, clamped in dough holders, and allowed different rest periods before being stretched. From a series of such extensograms the load at a constant sample deformation, corresponding to a kymograph extension of 7 cm. (corrected), was read and plotted against rest period to give the structural relaxation curve.

Each structural relaxation curve obtained in this study was analyzed in the manner previously described (4) to obtain the two constants characteristic of the relaxation curve. Regression coefficients were calculated for plots of the product of load × rest period *vs.* rest period. The intercept of such a plot gives the relaxation constant C, while the slope of the plot gives the asymptotic load L_A. The changes which take place in the physical properties of dough with reaction time can then be

[2] For the convenience of the reader, the optimum concentrations for each flour, expressed in p.p.m. (flour basis), are as follows: patent flour, 10 p.p.m. bromate, 3 p.p.m. iodate; intermediate flour, 15 p.p.m. bromate, 9 p.p.m. iodate; clear flour, 25 p.p.m. bromate, 24 p.p.m. iodate; germ flour, 15 p.p.m. bromate, 15 p.p.m. iodate. A concentration of 10 p.p.m. potassium bromate is equivalent to 0.36 microequivalents per g. of flour; a concentration of 3 p.p.m. potassium iodate is equivalent to 0.064 microequivalents per g. of flour.

characterized by plotting either the relaxation constant C or the asymptotic load L_A *vs.* reaction time.

Structural Relaxation Experiments: Results and Discussion

Figure 2 presents some of the structural relaxation data obtained in this study, and illustrates the relaxation curves obtained after reaction times of 5 minutes, 2 and 4 hours for untreated control doughs and for doughs containing the optimum level of potassium iodate or potassium bromate, for two of the four flours. These results show that there is a marked difference between the effects of these two improvers.

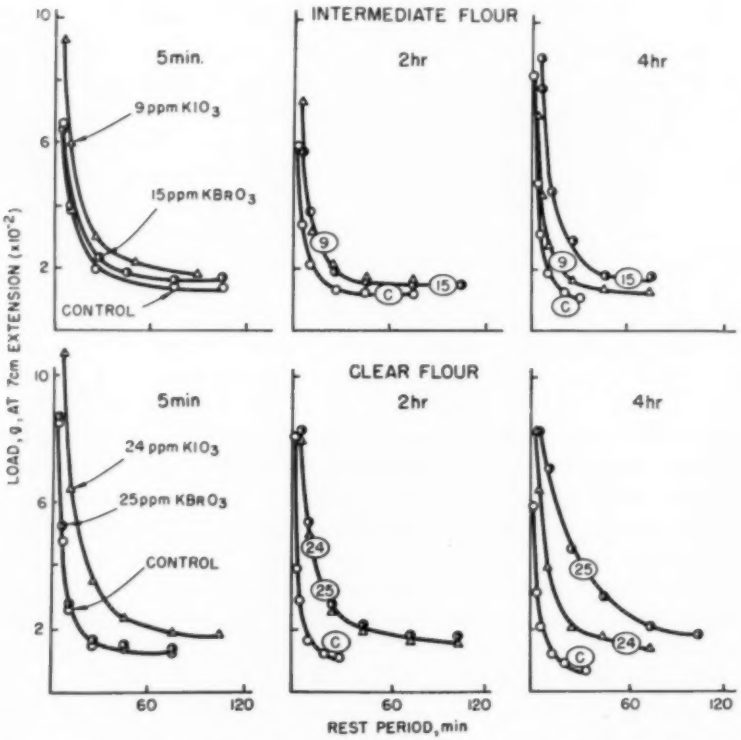

Fig. 2. Structural relaxation curves for control (○), optimally bromated (●), and optimally iodated doughs (△) for reaction times of 5 minutes, 2, and 4 hours: top, intermediate flour; bottom, clear flour.

Figure 2 shows, as has been noted from qualitative observations by Sullivan and co-workers (14), that the reaction of iodate in dough is rapid. Addition of iodate to dough produces a considerable change in

the relaxation curve for a reaction time of 5 minutes (left-hand graphs). Relaxation is somewhat slower in the iodate-treated dough than in the control. In addition, the curves for iodated doughs stabilize at higher load values than the corresponding controls. By comparison, there is very little difference between the 5-minute curves for bromated and control doughs.

With increasing reaction time (center and right-hand graphs), relaxation in untreated control doughs becomes more rapid and the relaxation curves fall progressively lower on the load axis. A similar trend is observed with increasing reaction time for the curves for iodate-treated dough. Thus the effect of iodate appears to be immediate, and there is no appreciable time-dependent response in dough; such further changes as occur in iodated doughs are similar to those occurring in untreated control doughs.

Unlike iodate, bromate in these concentrations does not have an appreciable immediate effect in dough; the reaction of bromate is time-dependent. Although the relaxation curves for bromate-treated and control doughs are very little different for a reaction time of 5 minutes, marked differences do gradually develop with increasing reaction time.

The effect of bromate on the clear flour (bottom row, Fig. 2) is similar to results previously published (2), and can therefore be dismissed briefly. With increasing reaction time, relaxation becomes progressively slower and at the same time the relaxation curve gradually rises on the load axis.

A somewhat different effect is observed for the intermediate flour (top row), presumably because the bromate concentration, at 15 p.p.m., is somewhat lower than that used in previous studies. In a reaction time of 2 hours, the relaxation curve for control doughs is displaced downwards, as is the curve for the bromated doughs, though to a much lesser extent. Thus there is a greater separation between the relaxation curves for the control and bromated doughs after a 2-hour reaction than there was after only a 5-minute reaction. After a reaction time of 4 hours, the curve for control doughs has been displaced still further downwards, whereas the curve for bromated doughs has moved in the opposite direction and is now definitely higher than the 2-hour curve and fractionally higher than the 5-minute curve. Thus the changes in the properties of bromated dough from intermediate flour seem to be affected by two opposing reactions: the bromate reaction, which tends to decrease the relaxation rate and at the same time raise the relaxation curve; and a reaction, which also occurs in control

dough, that tends to increase the relaxation rate and gradually lower the relaxation curve.

A more generalized description than the above qualitative discussion of structural relaxation in dough can be obtained by mathematical analysis of data (4). It is assumed that the relaxation curve can be closely approximated by the equation of an equilateral hyperbola referred to its asymptotes as axes. Two constants then serve to characterize the relaxation curve, the relaxation constant C which is a measure of the relative rate of structural relaxation in the dough, and the asymptotic load L_A which is the value the relaxation curve approaches at infinite rest period. The relaxation constant C seems to be a somewhat more sensitive indicator of the changes in dough properties produced by improvers and is therefore chosen for further examination.

Figure 3 is a plot of the relaxation constant, C, against reaction time, for control doughs and for doughs containing the optimum concentrations of bromate and iodate; separate graphs are shown for each

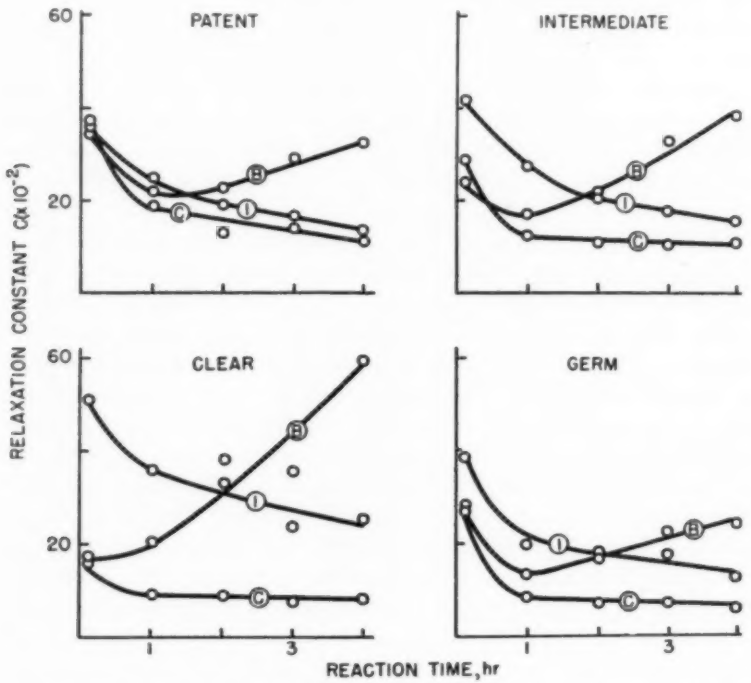

Fig. 3. Effect of reaction time on relaxation constants for control (C), optimally bromated (B), and optimally iodated doughs (I) from four flours.

flour. The relaxation constants for control doughs decrease as the reaction time is increased, i.e. relaxation becomes more rapid in agreement with previous observations (4). However, in the present study a rather much more marked drop in the relaxation constant is observed within the first hour of reaction time; subsequently, an almost linear decrease takes place in agreement with earlier results.

The changes in the relaxation constants of iodate-treated doughs with increasing reaction time reflect the rapid reaction of iodate in dough. Except in patent flour, iodate produces a marked increase in the relaxation constants for a reaction time of 5 minutes. The magnitude of these initial changes depends partly upon the iodate concentration and partly upon the grade of flour. With increasing reaction time, the curves for iodated doughs fall in a similar manner to those for control doughs.

The addition of bromate to dough produces changes in the relaxation constants that differ widely from those produced by iodate and which emphasize again the time-dependence of the bromate reaction. Bromate produces very little change in the relaxation constants for a reaction time of 5 minutes. However, gradual changes in the relaxation constant take place with increasing reaction time. At first, for patent, intermediate, and germ flours (containing the lower concentrations of bromate, 10 and 15 p.p.m.), the relaxation constant decreases appreciably but at a rate different from that for the controls. The rate constant passes through a minimum and then increases apparently linearly with time. For the clear flour, treated with the highest bromate level (25 p.p.m.), an increase in the relaxation constant across the whole range of reaction times was observed, similar to that found in previous studies (4) when the concentration of bromate was of this same order.

Figure 3 also emphasizes differences in the rheological behavior of dough made from different grades of flour. The decrease in the relaxation constant for control doughs during 1-hour reaction varies considerably with the flour grade, being least for the clear, considerably larger for the intermediate, and somewhat larger still for the patent and germ flour doughs. The slope of the linear portion of these curves for the control doughs is in a similar manner dependent upon flour grade.

As would be expected, the response of the different flours to improver treatment also varies. For example, the change in the 5-minute relaxation constant produced by 9 p.p.m. of potassium iodate in the intermediate flour dough was considerably larger than that produced

by 15 p.p.m. potassium iodate in germ flour dough. Again, for the same two flours, intermediate and germ, the responses to 15 p.p.m. potassium bromate were quite different, the increase over the linear portion of the curve being considerably higher for the intermediate flour. However, it is difficult to make comparisons between flours since the absorption varies from flour to flour, and it has been found in this laboratory that the relaxation behavior also changes considerably with changes in absorption.

The foregoing discussion related to "optimum" levels of improvers. Some additional studies were made at other levels of improvers, and these provide data for plotting the rate of the bromate reaction in unleavened dough against the bromate concentration (Fig. 4). For this purpose, the slopes of the linear portions of the curves in Fig. 3 (for control and bromated doughs), which are measures of the rate of change of physical dough properties, are taken as the rates of the bromate reaction. In addition to the data derived from Fig. 3 for the control and optimally bromated doughs, data are also included for a

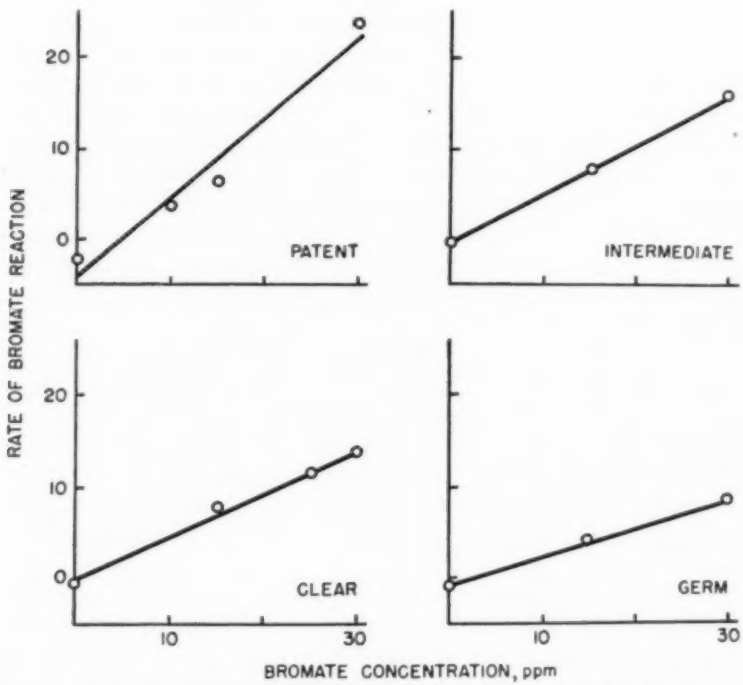

Fig. 4. Effect of concentration on the rheologically determined rate of bromate reaction in four flours.

level of 30 p.p.m. for all four flours and for 15 p.p.m. for the patent and clear flours.

The curves in Fig. 4 show that the rate of the bromate reaction in unleavened dough is a linear function of the bromate concentration within the range from 0 to 30 p.p.m. The dependence of the rate upon concentration varies with flour grade. It is greatest for the patent flour, followed in decreasing order by the intermediate, clear, and germ flours.

The present investigation suggests that a number of different reactions may well control the rheological properties of doughs. It was suggested earlier (2) that the changes in properties of untreated control doughs result from degradation of the dough structure. This now appears to involve at least two processes: the first results in the initial marked drop in the relaxation constant during the first hour (Fig. 3); the second results in the subsequent slow and almost linear decrease. Moreover, it appears that the changes in dough properties caused by bromate may be independent of the degradation process; bromate does not appear to prevent degradative rupture of the polymer network but to offset it by producing cross-linking at other points. All processes appear to take place simultaneously, the ultimate properties of the dough being controlled by the relative rates of the various reactions.

III. Chemical Analyses: Methods

The disappearance of potassium bromate and potassium iodate in unleavened doughs was studied by a modification[3] of the Cunningham and Anderson method for the determination of bromate in flour (1). Doughs, identical with those used in the rheological experiments, were mixed for 2.5 minutes in the G.R.L. mixer and placed in closed containers in a cabinet maintained at 30°C. Subsamples of 40 g. of dough were scaled from each parent at the end of certain desired reaction times. The reaction times employed were identical with those used in the rheological studies, i.e., 5 minutes, 1, 2, 3, and 4 hours.

Each subsample of dough was dispersed in 181.5 ml. of water by mixing for 3 minutes at high speed in the Waring Blendor (Model No. PB5). The blender was then turned to low speed and 25 ml. zinc sulfate solution (155.1 g. per liter) and 25 ml. sodium hydroxide solution (26.0 g. per liter) were added in succession to clarify the dispersion. Three drops of *N* octyl alcohol were added to break the foam. The mixture was then centrifuged at a relative centrifugal force of 1500 × G for

[3] Cunningham, D. K. Unpublished results.

10 minutes. Bromate or iodate was determined in the extract by adding, to a 20 ml. aliquot, 5 ml. of 0.001*N* sodium thiosulfate solution, 5 ml. of 10% sulfuric acid, and 3 ml. of 30% potassium iodide solution. Excess thiosulfate was back-titrated with 0.001 *N* potassium iodate. The end point was determined amperometrically (1).

Chemical Analyses: Results and Discussion

Preliminary experiments (See footnote 3) on the application of the Cunningham and Anderson method for potassium bromate to the determination of potassium iodate in flour and dough indicated marked differences in the reaction of these two improvers. It had been adequately demonstrated in previous studies that the reaction of bromate in dough was quite slow (7). The results of the present study show that the reaction of iodate, on the other hand, is very rapid.

Small amounts of iodate added to flour dough react immediately; no iodate can be recovered from the dough even when the determination is made immediately after the dough is taken from the mixer. When increments of iodate are added to dough, there is found to be a certain concentration above which iodate can be recovered from the dough when the determination is made immediately after mixing. This particular concentration, called the "iodate saturation value," is characteristic of the flour. For the four grades of flour used in this study, the iodate saturation values are recorded below.

Flour	*Iodate Saturation Value* μ*. equiv/g flour*
First patent	0.92
Intermediate	1.24
Clear	1.63
First germ	1.64

When excess iodate is added to dough, an amount equal to the saturation value is consumed or reacted very rapidly, but the remainder can be recovered. However, if a reaction time be allowed, there is a further progressive loss of iodate in the dough. This further reaction of iodate takes place at a readily measurable rate which appears to vary with the flour grade; the lower the grade of flour, the more rapid the rate of disappearance. This finding led to a more extensive investigation which will be reported in a later paper.

In contrast to the rapid reaction of iodate in dough, bromate at all levels of addition disappears very slowly from dough. The disappearance of potassium bromate in unleavened doughs prepared from each of the four grades of flour was followed by determining the amount of improver remaining after various reaction times in doughs which

were initially mixed to contain the optimum concentration indicated by the baking test. These results are recorded in the left-hand graph of Fig. 5 where the residual bromate, in micro-equivalents per g. flour, is plotted against reaction time. The values plotted for 0 time represent the amounts originally added to the doughs at the time of mixing.

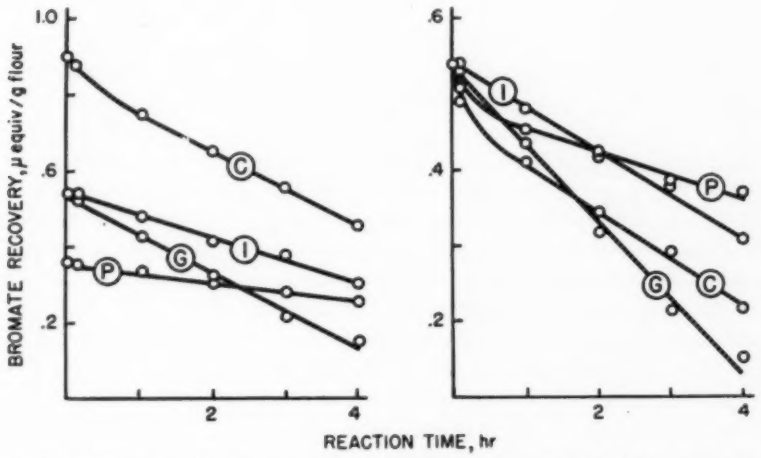

Fig. 5. Effect of reaction time on recovery of bromate from unleavened doughs for four flours: left, initial concentration equal to optimum; right, uniform initial concentration of 0.51 μ eq/g (15 p.p.m.).

Figure 5 (left) shows that the concentration of bromate in unleavened dough decreases at a uniform rate over a major portion of reaction time investigated. There is, however, a slight deviation from linearity for short reaction times for the clear flour, as well as a suggestion of a similar deviation for the patent and germ flours. These curves show that there is an appreciable difference in the rate of disappearance of the optimum bromate level from the doughs of the four flours.

These differences between flours are shown more clearly by the data in the right-hand graph of Fig. 5, for which all doughs were mixed to contain 15 p.p.m. of bromate. Here again there is evidence for a rapid but slight disappearance of bromate, for both the patent and clear flours, at the beginning of the reaction period. The initial phase of the bromate reaction in the patent flour is magnified somewhat in Fig. 5 (right) over that shown in Fig. 5 (left) by the use of a higher bromate concentration. For a constant initial level of added improver, bromate disappears most rapidly from the germ flour.

Bromate loss in intermediate and clear flour doughs occurred at a rate about 50% of that for the germ flour doughs, whereas in patent flour dough the rate was about 25% of this maximum.

Concurrent studies in this laboratory (footnote 3), which will be published separately, have shown that the rate of disappearance of potassium bromate in leavened dough may differ from that in unleavened dough.

General Discussion

Each of the foregoing sections of this paper has described the effects of the reactions of bromate and iodate in dough as observed by a particular method of measurement. Each of these criteria of measurement provides information at a different level of abstraction about the reaction of these improvers. The chemical analyses provide the most specific information of all three methods, but this information is restricted to the improver; no information about the dough or flour constituents which are also involved in the improver reaction is available from the analytical results. At the other extreme, the baking test is the least specific of the methods of measurement, since the baked loaf represents a summation of all the complex biological, physical, and chemical interactions that have taken place during the making of the bread. The study of the rheological behavior of dough provides information intermediate between these extremes. The relaxation curves reflect the over-all changes taking place in unleavened dough as a result of the reaction of added improvers as well as of the reaction of certain constituents of the flour itself. It may be argued that the results of the rheological study are limited in scope in the sense that the reactions of the two improvers were followed in unleavened rather than leavened dough. Nevertheless, this study does yield some data about the reactions of bromate and iodate in dough which must be accounted for by any hypothesis of improver reaction.

Each of the individual methods of measurement used in this study has indicated rather marked differences between the effects in dough of potassium bromate and potassium iodate. Equivalent concentrations of these improvers did not produce equal effects in the baked bread. Optimum loaf properties produced by iodate occurred at lower equivalent concentrations than did the optima for bromate. Furthermore, the magnitude of improvement was considerably less for iodate than for bromate for two of the four flours used. Both rheological and chemical measurements indicated a marked difference in the rates of reaction of these improvers in dough. Iodate reacts very rapidly, bromate quite slowly.

It is of interest to compare some of the results of the three individual criteria of measurement in an attempt to determine if any apparent relations exist between the various manifestations of improver reaction. An attempt has been made to make the desired comparisons graphically (Fig. 6), presenting, first, some of the relations pointed up by the baking tests, then some of the interrelations between the various methods of measurement.

It is emphasized, however, that this part of the investigation must be considered only as a preliminary study. Definite conclusions on the basis of the comparisons to be made are probably unjustified. Rather, the results may be considered to indicate certain areas worthy of more detailed study.

Figure 6, A, is a plot of loaf volume vs. protein content of the flour for untreated doughs and for doughs containing the optimum concentrations of bromate and iodate. For each separate condition, loaf volume tends to change regularly with protein content, though the points for germ flour are out of place. The curves indicate that the optimum response of flour to bromate may be quite different from the response to iodate. The negative slope for the curve for control doughs is probably accounted for by the fact that decreasing flour grade was accompanied by an increase in protein content. Again, in Fig. 6, B, which is a plot of the optimum loaf volume against the optimum improver dosage, the results for the germ flour do not conform to the trends indicated by the other three flours. With the exception of the germ flour, optimum loaf volume is roughly a linear function of improver dosage. The marked difference between the effects of bromate and iodate is again indicated.

In the remaining graphs of Fig. 6, certain of the rheological or analytical manifestations of improver action are compared with the baking results in an attempt to determine which factors are likely to be most significant in determining baking behavior. Figure 6, C, is a plot of the relaxation constant for a reaction time of 3 hours for unleavened doughs against the loaf volume for corresponding leavened doughs. This reaction time in unleavened dough was selected because it corresponds most closely to the reaction (i.e., fermentation) time allowed in the baking test. These data suggest that there may be a significant relation between the rheological behavior of unleavened dough and the baking behavior of the leavened dough. The data for both the control and bromated doughs suggest a linear relation. The incorporation of bromate produces a shift in the curve, but the two curves appear parallel. The effect of flour grade in this relation is not

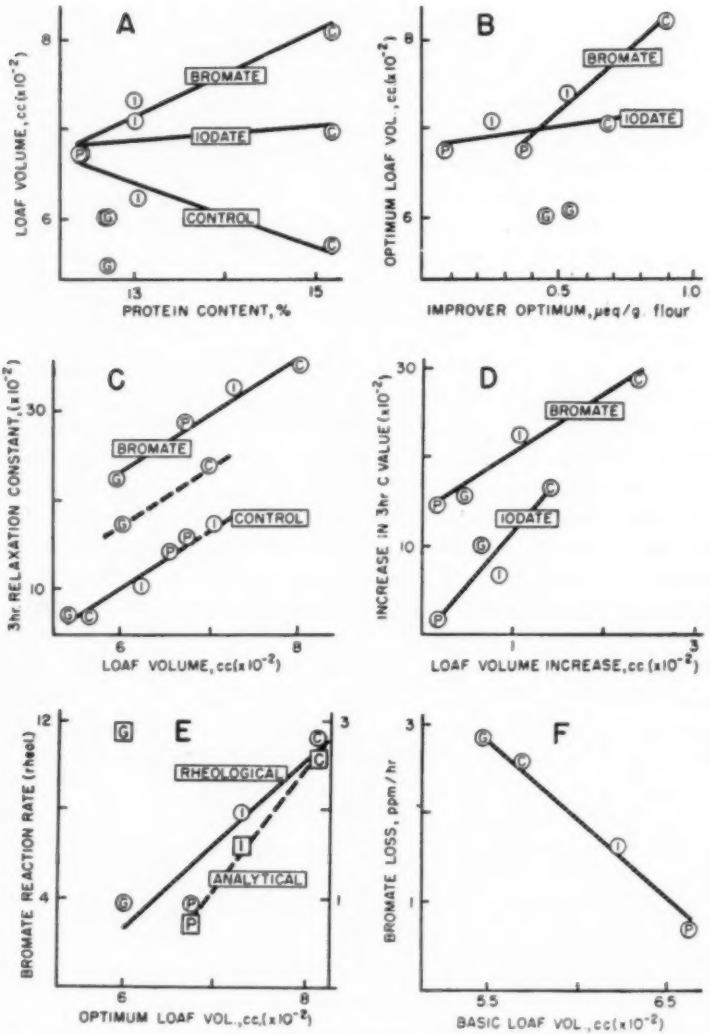

Fig. 6. Comparison of various manifestations of improver action in patent (P), intermediate (I), clear (C), and germ flours (G): A, relation between loaf volume and protein content for control, optimally bromated, and optimally iodated doughs; B, relation between optimum loaf volume and optimum improver dosage; C, relation between magnitude of 3-hour relaxation constant for unleavened dough and loaf volume; D, relation between increase in relaxation constants at 3 hours and increase in loaf volume produced by reaction of optimum level of bromate or iodate; E, relation between rate of bromate reaction in unleavened dough and optimum loaf volume produced by bromate: circles, reaction rate from rheological measurements; squared points, analytically determined rate of bromate loss; F, relation between rate of bromate loss in unleavened dough and basic loaf volume.

clear, for, while the increase in loaf volume for the control doughs corresponds to increasing flour grade, such is certainly not the case for the bromated doughs. The effect of iodate as shown in this representation is not simple. The incorporation of iodate, in the higher-grade patent and intermediate flours, yields data which fall on an extrapolation of the curve for control doughs, whereas points for iodate-treated clear and germ flours lie intermediate between the curves for control and bromate-treated doughs. A line through these two points is parallel to the other two curves.

Two further attempts to discover a relation between changes in physical properties of unleavened dough and baking behavior are shown next. Figure 6, D, suggests that the increase in the 3-hour relaxation constant produced by adding the optimum concentration of improver may be closely related to the corresponding increase in loaf volume in the baking test. This change in the relaxation constant represents the magnitude of the changes in dough properties produced in unleavened dough by the reaction of improvers in a time corresponding to the fermentation time used in the baking test. A marked difference between bromate and iodate is reflected in this representation.

Figure 6, E, is a plot of the rate of the bromate reaction in unleavened dough against optimum loaf volume. On the one hand (circles), the rate of the bromate reaction is determined from rheological experiments as the slope of the linear portion of the plot of relaxation constant vs. reaction time for bromated doughs (Fig. 3). On the other hand (squared points), the rate of the bromate reaction is the analytically determined rate of bromate disappearance in optimally bromated unleavened doughs. The data for all four flours suggest that optimum loaf properties may be closely related to the changes which bromate produces in the rheological behavior of unleavened dough. However, because the point for the germ flour is so far removed, it does not seem as likely that the rate of bromate disappearance is so closely related to optimum loaf properties. Rather it would appear from Fig. 6, F, where the rate of bromate disappearance is plotted against the basic loaf volume, that the disappearance of bromate is closely related to flour grade.

A logical way of harmonizing these observations of the reactions of bromate and iodate in dough is to assume that the improving effect of various reagents arises, not from a single specific chemical reaction, but rather from different chemical reactions. This hypothesis is consistent with much technological knowledge of dough and bread prop-

erties. Doose and Wolter (5), for example, demonstrated that by appropriately varying any one or a combination of two or more of several conditions during processing, an improvement in loaf properties can be effected. Moreover, this hypothesis is also consistent with the concept of a three-dimensional or network structure in dough. It seems reasonable that in dough, with its complex composition, different reagents cause different chemical reactions which produce changes in the degree of cross-linking of the network. The results of this study suggest that the optimum loaf volume produced by treating a flour with a given improver occurs with the attainment of a particular, but not necessarily specific, combination of dough properties, at a specific time in the bakng schedule, i.e., at the time of molding.

The foregoing comparison of the action of the improvers bromate and iodate has provided suggestive data which must be considered as qualitative only. The results of this study do, however, serve to indicate certain areas where more detailed study would be expected to yield more specific information on some of the individual factors involved in the mechanism of improver reaction.

Acknowledgment

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THE PENETRATION OF UREA AND ORTHOPHOSPHATE INTO THE WHEAT KERNEL[1]

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ABSTRACT

Wheat kernels infiltrated under various conditions with rat urine containing C^{14} as urea or P^{32} as inorganic phosphate and, in some cases, with indigo carmine were embedded in celloidin. After hardening, histological sections were cut and exposed to autoradiographic plates by direct contact. Penetration of P^{32} and C^{14} into sound kernels, as well as into kernels with hair-line cracks in the surface, occurred particularly around the germ and along the crease. Washing the infiltrated kernels reduced the radioactivity on the surface but did not affect the contamination in the interior.

Urea and radioactivity determinations were made on the various mill fractions obtained on the Buhler experimental mill from a commercial hard red spring wheat tempered for milling with solutions containing orthophosphate-P^{32} and urea in concentrations corresponding to those found in rat urine. They showed that while bran presents a considerable barrier to their entry, both constituents penetrated the endosperm at different rates and contaminated the flour. Thus, with P^{32} the bran contained 92.6%, the shorts 4.2%, and the flour streams 3.2% of the total activity; of the total urea, 67.7% was found in the bran, 7.6% in the shorts, and 24.7% in the flour streams. Washing the kernels at 28°C. for 30 seconds with vigorous agitation removed about 60% of the total urea present, but the loss was almost entirely from the bran layers. Neither dry-scouring the kernels nor washing them prior to milling had any appreciable influence on the total radioactivity or urea content of the flour fractions. Because of the high level of contamination employed, the uniformity with which the solutions of orthophosphate and urea were distributed among the kernels, and the short experimental milling system used in these experiments, the degree of penetration observed cannot be interpreted as a measure of the extent to which flour might be contaminated in commercial milling practice.

In recent years increasing emphasis has been placed on improving the sanitation of wheat. The comprehensive survey of insect and rodent contamination of wheat grown in Montana, North and South Dakota, and Minnesota conducted by Butler and Mickel (2) showed that rodent contamination of wheat was somewhat more extensive than was infestation with insects. The presence of rodent fecal pellets is indicative that the wheat is also contaminated with rodent urine and there is need for information on the extent to which the urine will spread over the surface of the kernels and penetrate into the endosperm.

Contamination of wheat by rodent urine has been under study in these laboratories. Laakso *et al.* (8) have recently reported the results of some limited experiments on the number of wheat kernels which gave positive tests for urea when adult rats are allowed to roam over

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the surface. The extent to which rodent urine can penetrate the outer layers of the wheat kernel and gain access to the endosperm is of particular importance in relation to production of wheat flour. The investigations which the authors have been conducting on this subject are reported in the present paper in two parts. The first comprises qualitative studies involving the preparation of autoradiographs from histological sections of wheat kernels after treatment with urine containing inorganic phosphate-P^{32} and urea-C^{14}. The second part deals with quantitative studies on the distribution of urea and inorganic phosphate-P^{32} in the various fractions obtained in the Buhler experimental mill from a hard red spring wheat which was tempered for milling with a solution of urea and inorganic phosphate-P^{32}. The effect of dry-scouring and washing the wheat before milling on the distribution of these contaminants was studied.

Qualitative Studies on Penetration of Orthophosphate-P^{32} and Urea-C^{14} into Wheat Kernels

The extent of penetration of urinary constituents into different parts of the wheat kernel may be studied qualitatively through application of radioactive tracers present in two compounds, inorganic phosphate and urea, which are normal constituents of rodent urine. Techniques were developed for the preparation of microscopic sections of wheat kernels which had been infiltrated with rat urine containing C^{14} as urea or P^{32} as inorganic phosphate. By making a series of sections through the kernel and using the same sections for microscopic observation as for the preparation of autoradiographs, it was possible to secure qualitative information on the extent of penetration of inorganic phosphate and urea into different portions of the kernel. Many trials had to be made to determine the most suitable concentrations of radioactive compounds to use, to develop a technique for the prevention of "flouring" of the kernel during the cutting of very thin sections, and to find satisfactory procedures for the preparation of histological sections and autoradiographs. Kernels with exposed germs or with hair-line checks or cracks in the surface would be expected to show greater penetration of urinary constituents than sound kernels. Kernels of these three types as revealed by visual and X-ray examination[2] were included.

Materials and Methods

Preparation of Kernels Infiltrated with Urea-C^{14}. Sound wheat kernels were soaked for 4 hours in 0.5 ml. normal rat urine containing

[2] Courtesy of the Department of Entomology and Economic Zoology, University of Minnesota, and of Pillsbury Mills, Inc., Minneapolis, Minnesota.

approximately 45 micromoles of urea-C^{14} equivalent to 45 microcuries of C^{14}. On removal from the infiltrating solution, the kernels were divided into two groups, designated "washed" and "unwashed." "Unwashed" kernels were blotted with paper toweling, air-dried, and then placed in celloidin (30% solution of cellulose nitrate dissolved in ethyl ether-absolute alcohol, 1 + 1) to embed. "Washed" kernels were washed briefly three times with a thin stream of distilled water, drained on paper toweling, and then air-dried.

Preparation of Kernels Infiltrated with Orthophosphate-P^{32}. Two series of tests were made using different periods of immersion of the kernels. For the first series, penetration studies were made with kernels from three lots of wheat which were previously subjected to X-ray analysis: lot 1, sound spring wheat (grade 1 HDN); lot 2, hard spring wheat containing checks in the seed coat; and lot 3, mixture of hard red winter and hard red spring wheats containing germs which were exposed.

Kernels from each of the samples were soaked for 24 hours in 1 ml. normal rat urine containing 0.1 ml. of 0.15*M* phosphate buffer (pH 7.4), 2 mg. indigo carmine[3], and 0.01 ml. of 0.25*M* orthophosphoric acid-P^{32} solution equivalent to 20 microcuries of P^{32}. After removal, the kernels were washed three times briefly with a thin stream of distilled water, drained on paper toweling, and air-dried.

For the second series, sound wheat kernels from lot 1 were soaked for 30 minutes in 1 ml. rat urine containing 0.1 ml. of 0.15*M* phosphate buffer (pH 7.4), 2 mg. indigo carmine, and 5.6 μl. of 0.25*M* orthophosphoric acid-P^{32} containing 15 microcuries of P^{32}. On removal, kernels were blotted with paper toweling, and air-dried.

Preparation of Sections and Autoradiographs. The air-dry kernels were embedded with celloidin thinned to the proper consistency with a mixture of ethyl ether and absolute ethanol (1 + 1), for 1 to 2 weeks in a screw-top jar. To begin the hardening procedure, the cover was partially removed from the jar. When the celloidin was sufficiently thick owing to evaporation of the solvent, each kernel was oriented in the celloidin in such a way as to permit subsequent sectioning of the kernel, either parallel or perpendicular to the crease. The hardening process was continued by placing the uncovered jar in an atmosphere of chloroform until the celloidin was hard enough to permit removal of a block approximately 1 by 1 by 2 cm. containing the kernel. The block was immediately placed in a mixture of 70% ethanol, 20% water, and 10% glycerol. The block was mounted by moistening one

[3] Indigo carmine was used here in connection with concomitant studies of the penetration of certain colored substances into the wheat kernel.

side with ethyl ether-absolute ethanol (1 + 1) and then placing it firmly on a metal mount containing some wet celloidin on its surface. The mounted block was either sectioned at 40 μ immediately or stored by submersion in the aqueous ethanol-glycerol solution until sectioned. During the entire sectioning procedure it was necessary to keep the block, knife, and section wet with the alcohol-glycerol solution to prevent the sections from curling or becoming brittle. For the mounting procedure, glass slides 1 inch by 3 inches were painted evenly with a 3% solution of gelatin and allowed to dry. Four to six successive sections at each of four or five areas through the kernel were transferred from the microtome blade to the gelatin side of the glass slide with a brush. As each slide was prepared, it was covered with a 1-inch by 3-inch strip of filter paper on which was noted the approximate numerical order of the sections and the identification of the kernel. Another slide was secured over the filter paper and attached by tape to the slide containing the sections. The alcohol was then allowed to evaporate so that the sections would become securely attached to the gelatin-covered slide. After 24 hours the tape was removed, and the slide containing the sections was then placed in intimate contact with an Eastman autoradiographic plate A (25 μ + T coating, 1 inch by 3 inches) in the dark room. The two slides, autoradiographic plate and the one containing the histological sections, were secured by tape and exposed in a closed dark refrigerator for the time required to secure a satisfactory autoradiographic image. The sections from kernels infiltrated with urea-C^{14} were exposed for 14 to 16 weeks. Those from the first series infiltrated with orthophosphate-P^{32} were exposed for 3 days beginning two half-lives after infiltration; the second series was exposed 10 days beginning 3.5 half-lives after infiltration. After sufficient exposure the autoradiographs were developed.

Leaching Effects. Visual inspection of kernels infiltrated with P^{32} during the sectioning process of the first experimental series showed that the indigo carmine dye had penetrated all kernels well. Some leaching of the blue color was seen in the solutions used to wet the knife and the celloidin block. Some blue color was also visibly streaked through the block at several points; possibly this was leached from the kernel when it was oriented in the thickening celloidin. Radioactivity was detected in the celloidin independent of the presence of the dye.

Results

Comparisons of the autoradiographs with the histologic sections under the microscope showed that inorganic phosphate-P^{32} and

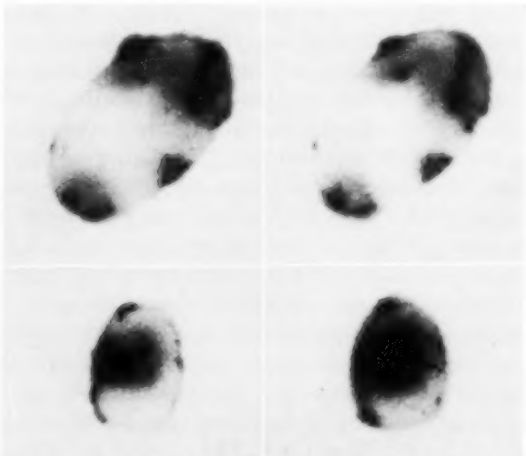

Fig. 1. Photographs of autoradiographs made from histologic sections of wheat kernels soaked for 24 hours in normal rat urine containing 20 microcuries of P^{32} as orthophosphate and 2 mg. indigo carmine. [Indigo carmine was used in connection with concomitant studies of the penetration of certain colored substances into the wheat kernel.]

Top row: Sections from a kernel of sound hard red spring wheat, grade 1 Dark Northern. Sections cut parallel to the crease of the kernel.

Bottom row: Sections from a kernel of the same type wheat as above but the kernel was sectioned perpendicular to the crease. Note that intense concentration of radioactivity in the crease caused blurring of the section outline, particularly in the crease area.

urea-C^{14} penetrated sound as well as checked kernels, particularly around the germ and along the crease. While washing the kernels after they were soaked in urea-C^{14} for 4 hours removed some of the urea from the surface, it had no apparent influence on the degree of contamination in the interior. Photographs of representative autoradiographs and histologic sections are shown in Figs. 1 and 2. Urea-C^{14} gave more contrasty negatives than phosphate-P^{32}.

Quantitative Studies on the Distribution of Orthophosphate-P^{32} and Urea in Mill Streams Obtained in the Buhler Mill from Wheat Tempered with a Solution of Orthophosphate-P^{32} and Urea

To secure a measure of the extent to which urinary constituents can become distributed in the wheat kernel and contaminate the flour, experiments were performed in which a commercial wheat was tempered with a solution of inorganic phosphate-P^{32} and urea solution and milled in the Buhler mill; the various mill fractions were weighed and samples analyzed for radioactivity and urea content, as well as for moisture, protein, and ash content. Two series of experiments were made. In the first, a test was made of the replicability of the procedure and in the second, the influence of dry-scouring and of wet-washing the wheat was investigated.

Fig. 2. Photographs of autoradiographs made from histologic sections of sound hard red spring wheat kernels soaked for 4 hours in normal rat urine containing 45 microcuries of C^{14} as urea.
Top row: Sections cut parallel to the crease of a kernel (grade 1 Dark Northern) which was not washed on removal from the C^{14} urine solution. Before the kernel was embedded with celloidin it was merely air-dried.
Bottom row: Sections cut perpendicular to the groove of a kernel which was not washed on removal from C^{14}-urine solution, before it was embedded with celloidin.

Materials and Methods

Preparation of Samples for Milling. In the first experimental series, 2000 g. of wheat (No. 1 Dark Northern Spring), 11.5% moisture, were mixed with 100 ml. of a solution containing 0.05 *M* phosphate-P^{32} (pH 7.4) containing 5.0 g. of urea. The solution was added slowly from a buret while the wheat was slowly stirred mechanically. The sample was stored for 18 hours in a closed container with occasional shaking to bring it into proper condition for milling.

A second series of experiments was made to secure a measure of the influence of dry-scouring and of washing to which wheat is normally subjected commercially before it is milled. To 5798 g. of the commercial wheat mix at 11.5% moisture were added 300 ml. of the solution of 0.05 *M* inorganic phosphate-P^{32} containing 15 g. of urea, which brought the moisture content to 16.0%. The mixture was held at room temperature in a closed container for 72 hours and then dried to 12.4% moisture in an air oven maintained at 37.8°C. The wheat was held 48 hours in a closed container to permit the moisture distribution within the grains to approach equilibrium. The sample was then divided into three lots of 1953 g., each of which was treated as follows:

Lot 1 was tempered to 15.5% moisture, allowed to stand in a closed container for 18 hours, and then milled.

Lot 2 was passed through a Wolf experimental scourer once, tempered to 15.5% moisture, held for 18 hours, and then milled.

Lot 3 was washed with a strong stream of water at 28°C. with vigorous agitation, allowing the water to flow through the grain for 30 seconds, after which the wheat was transferred to a Büchner funnel and vacuum applied to remove the excess water. The elapsed time from the start of the washing process to the completion of filtration was 5 minutes. The wheat was dried to 14.6% moisture at 37.8°C., tempered to 15.5% moisture, held in a closed container for 18 hours, and then milled.

Experimental Milling. Milling tests were carried out in a Buhler automatic experimental mill. A preliminary sample of a tempered wheat treated to the same level of radioactivity as the experimental series was put through the mill. The sieve boxes were then emptied but the mill was not cleaned out before the experimental samples were milled. After about one-third of each wheat had passed through, the sieve boxes were emptied and the mill fractions obtained during the remainder of the operation were collected and weighed. The yields of each fraction were computed on the basis of the total weights of material recovered. This procedure was followed in order to avoid undue contamination of the mill room and personnel with radioactive materials. After each series of tests, the mill was allowed to stand idle until the radioactivity had fallen to low levels.

Methods of Analysis. Moisture, protein, and ash were determined on samples of the tempered wheats and the various mill fractions by the conventional procedures described in *Cereal Laboratory Methods* (1). Before analysis, the wheats, bran, and shorts were ground in a micro Wiley mill to pass a 40-mesh sieve. For measurement of radioactivity, 10 g. of each fraction and of the ground whole wheat were compressed in a Carver hydraulic press into a cylindrical pellet 3 cm. in diameter and approximately 1.5 cm. high. Fragmentation of the pellets during handling was prevented by coating the surfaces with du Pont Krylon acrylic spray. The radioactivity of each pellet in counts per minute above background was determined by conventional techniques using a Geiger-Mueller thin-windowed tube and a suitable scaler. Urea was determined on the pellets employing an adaptation of the method described for feeds in *Cereal Laboratory Methods.* The bran and shorts fractions were ground in a micro Wiley mill to pass a 40-mesh sieve and urea was determined on a 0.5-g. sample. After

digestion with urease solution at pH 4.5, a 5-ml. aliquot was transferred to a micro-Kjeldahl steam distillation apparatus, 1 ml. each of 25% calcium chloride solution and of 10% sodium hydroxide solution was added, and the ammonia collected in 10 ml. of 2% boric acid solution containing methyl red and bromcresol green as a mixed indicator. The distillate was collected for 3 minutes, and after an allowance of 1 minute for drainage of the condenser, the ammonium borate formed was titrated with 0.01 *N* hydrochloric acid solution. Fractions milled from untreated wheat were analyzed to serve as blanks; reagent blanks were also run. Trials with uncontaminated bran to which urea solution was added in an amount equal to that found in the bran from urea-treated wheat gave 95% recovery. Complete recovery of ammonia from a standard solution of urea digested with urease was obtained by the above procedure for distillation and titration of the ammonia formed.

Results

The results of the preliminary trial to test the replicability of the data obtained with orthophosphate-P^{32} are summarized in Table I. The duplicate trials with quite different levels of P^{32} are in good relative agreement. The bran possessed 131 and 150 times greater radioactivity per unit weight than the flour fraction of lowest activity (second- or third-reduction flour). When comparisons are made on the basis of the total weights of the various fractions, the bran contained 90.6 and 92.0% of the total activity, the shorts 5.5 and 6.6%, the break flours 1.1 and 1.3%, and the reduction flours 1.4 and 1.6%. Summation of the P^{32} values for all the flour fractions shows that 2.5 and 2.9% of the total radioactivity would be present in the straight-grade flour (ash content 0.42%, test 2).

The results of the second series of experiments designed to study the influence of dry-scouring and washing on the distribution of inorganic phosphate and urea are recorded in Tables II to IV inclusive.

The mill yields and chemical analyses for the control, dry-scoured, and washed samples in Table II are in good agreement. Total flour yields of 64.6 to 66.1% with ash contents of 0.40 to 0.44% were obtained. Although the flour yields are much lower than those for commercially milled straight-grade flours, the ash contents are quite similar.

The effects of dry-scouring and washing on the distribution of P^{32} and urea are shown in Tables III and IV. The radioactivity of the bran of the control sample per unit weight was approximately 100 times greater than that of the second-reduction flour; in contrast, the

TABLE I

DISTRIBUTION OF P^{32} IN MILL FRACTIONS FROM WHEAT TEMPERED WITH RADIOACTIVE PHOSPHATE SOLUTION[a]

FRACTION	YIELD	P^{32} PRESENT C.P.M. IN 10 G. SAMPLE	RELATIVE RADIOACTIVITY	TOTAL WEIGHT	EQUIVALENT C.P.M. FOR TOTAL SAMPLE	TOTAL P^{32} IN FRACTION	
	%			g.		%	
	Test 1 — April 3, 1954						
Bran	26.9	69,400	149.6	391	2,710,000	92.0	
Shorts	7.3	15,190	32.7	106	161,000	5.5	
Flours							
First break	5.4	1,860	4.0	78	14,500	0.5	
Second break	9.5	1,160	2.5	138	16,000	0.5	1.1
Third break	1.5	1,985	4.3	22	4,370	0.1	
First reduction	23.5	608	1.3	342	20,800	0.7	
Second reduction	11.2	464	1.0	162	7,520	0.3	1.4
Third reduction	14.7	595	1.3	214	12,700	0.4	
Totals	100.0			1,453	2,946,890	100.0	2.5
	Test 2 — Aug. 6, 1954						
Bran	25.5	8,520	131.1	384	327,000	90.6	
Shorts	8.4	1,890	29.1	126	23,800	6.6	
Flours							
First break	5.1	227	3.5	77	1,750	0.5	
Second break	9.6	155	2.4	144	2,230	0.6	1.3
Third break	1.5	282	4.3	22	620	0.2	
First reduction	24.2	84	1.3	364	3,060	0.9	
Second reduction	8.6	65	1.0	130	845	0.2	1.6
Third reduction	17.1	68	1.0	258	1,750	0.5	
Totals	100.0			1,505	361,055	100.1	2.9

[a] Protein and ash values on a 14% moisture basis for the flours obtained in Test 2 were as follows:

Flour	*Protein*	*Ash*
First break	13.7	0.52
Second break	15.3	0.55
Third break	17.2	0.62
First reduction	12.1	0.39
Second reduction	11.7	0.39
Third reduction	11.8	0.35
Summary		
All break flours	15.0	0.55
All reduction flours	11.9	0.38
All flours	12.7	0.42

relative activities of the bran from the dry-scoured and washed samples were 74 and 49 times greater respectively than the second-reduction flour. Dry-scouring slightly decreased, and washing greatly decreased, the total radioactivity of the bran fraction; however, these treatments had little effect on the total radioactivity of the flour fractions. Thus, the bran from the washed wheat gave a total of approximately 96,000 counts, and that from the dry-scoured wheat about 198,000 counts, in comparison with about 216,000 counts for the bran from the control.

TABLE II

YIELD, PROTEIN, AND ASH CONTENTS OF STREAMS OBTAINED IN MILLING THE CONTROL, DRY-SCOURED AND WASHED WHEATS ON THE BUHLER MILL AFTER TREATMENT WITH ORTHOPHOSPHATE (CONTAINING P^{32}) AND UREA SOLUTION[a]

FRACTION	YIELD			PROTEIN CONTENT			ASH CONTENT		
	Control	Dry-Scoured	Washed	Control	Dry-Scoured	Washed	Control	Dry-Scoured	Washed
	%	%	%	%	%	%	%	%	%
Wheat	...	...	...	14.0	13.7	13.6	1.44	1.41	1.29
Bran	28.1	27.6	27.5	17.5	17.2	16.7	4.96	4.81	4.30
Shorts	7.3	6.9	6.4	14.2	14.3	14.2	1.80	1.83	1.72
Flours									
First break	5.0	4.9	4.8	14.2	13.9	13.9	0.46	0.50	0.42
Second break	9.0	8.9	8.6	15.3	15.2	15.4	0.42	0.54	0.47
Third break	1.4	1.3	1.3	17.3	17.2	17.2	0.68	0.59	0.59
First reduction	24.8	27.0	28.0	12.1	12.0	12.1	0.38	0.36	0.36
Second reduction	9.0	8.6	9.7	11.8	11.7	11.9	0.42	0.38	0.37
Third reduction	15.4	14.8	13.7	11.9	11.9	12.5	0.37	0.33	0.38
Totals	100.0	100.0	100.0						
All break flours	15.4	15.1	14.7	15.1	14.9	15.1	0.46	0.53	0.46
All reduction flours	49.2	50.4	51.4	12.0	11.9	12.2	0.38	0.35	0.42
All flour	64.6	65.5	66.1	12.7	12.6	12.9	0.40	0.44	0.44

[a] All analyses are given on a 14% moisture basis.

TABLE III

INFLUENCE OF DRY-SCOURING AND WASHING ON THE P[32] ACTIVITY AND UREA CONTENT OF BUHLER MILL FRACTIONS OBTAINED IN MILLING HARD RED SPRING WHEAT TEMPERED WITH A SOLUTION CONTAINING ORTHOPHOSPHATE-P[32] AND UREA

Fraction	P[32] Activity Counts per Minute per 10 g.			Urea Content		
	Control	Dry-Scoured	Washed	Control	Dry-Scoured	Washed
				mg/g	*mg/g*	*mg/g*
Wheat (ground)	1255	1285	735	2.82	2.82	1.50
Bran	5275	4875	2325	5.88	5.43	1.34
Shorts	915	1165	615	2.48	2.62	1.75
First break flour	158	166	94	0.77	0.62	0.70
Second break flour	127	127	79	1.28	1.22	1.12
Third break flour	181	206	152	2.04	2.27	0.20
First reduction flour	63	73	50	0.57	0.33	0.49
Second reduction flour	54	66	47	0.98	1.04	0.98
Third reduction flour	53	73	51	1.27	1.46	1.03

TABLE IV

COMPARATIVE EFFECTS OF DRY-SCOURING AND WASHING WHEAT TREATED WITH RADIOACTIVE ORTHOPHOSPHATE AND UREA SOLUTION ON THE RELATIVE DISTRIBUTION OF P[32] AND UREA IN THE PRINCIPAL PRODUCTS OBTAINED ON THE BUHLER MILL

Fraction	Amount in Fraction[a]					
	Control		Dry-Scoured		Washed	
	P[32]	Urea	P[32]	Urea	P[32]	Urea
		g.		*g.*		*g.*
Bran	216.3	2.41	198.4	2.21	95.6	0.55
Shorts	9.8	0.27	11.9	0.27	5.8	0.17
Break flours	3.2	0.26	3.2	0.25	2.0	0.23
Reduction flours	4.2	0.62	5.3	0.58	3.8	0.56
All flours	7.4	0.88	8.5	0.83	5.8	0.79
All fractions	233.5	3.56	218.8	3.31	107.2	1.51
	Percent of Total P[32] or Urea in Fraction					
	Control		Dry-Scoured		Washed	
	P[32]	Urea	P[32]	Urea	P[32]	Urea
Bran	92.6	67.7	90.7	66.8	89.2	36.4
Shorts	4.2	7.6	5.4	8.2	5.4	11.3
Break flours	1.4	7.3	1.5	7.6	1.9	15.2
Reduction flours	1.8	17.4	2.4	17.5	3.5	37.1
All flours	3.2	24.7	3.9	25.1	5.4	52.3
All fractions	100.0	100.0	100.0	100.1	100.0	100.0

[a] The total P[32] activity and urea content of each fraction was calculated from total recoveries of 1461, 1474, and 1491 g., respectively, in milling the control, dry-scoured, and washed wheats and the percentage yields of each fraction recorded in Table II. The total recovery of urea in the mill products was 97.5, 89.7, and 40.5% respectively, for the control, dry-scoured, and washed wheats. The amounts of P[32] in each fraction are given as thousands of counts per minute.

In contrast, the total flour from the washed, dry-scoured, and control wheats gave approximately 5800, 8500, and 7400 counts respectively (Table IV). As a result of this differential effect of these premilling treatments on the P^{32} content of the bran as compared with the interior portions of the kernel, these treatments increased the percentage of total radioactivity present in the flour fractions. Thus, the straight-grade flours from the control, dry-scoured, and washed wheats contained 3.2, 3.9, and 5.4% of the total radioactivity of the various fractions.

Urea penetrated the bran much more readily than inorganic phosphate, with the result that it is more uniformly distributed throughout the various fractions. Thus, the straight flour from the control sample contained 24.7% of the urea added to the wheat, whereas it contained only 3.2% of the total radioactivity due to P^{32}. Washing, and to a much lesser extent, dry-scouring, reduced the urea content of the bran. Thus, calculations show that while 97.5% of the urea in the control wheat was recovered in the mill products, 89.7 and 40.5% respectively were recovered from the wheats which were dry-scoured and washed. As with P^{32}, however, these sample treatments had no significant influence on the amount of urea found in the flour fractions. The straight-grade flour (all flour fractions) from the control, dry-scoured, and washed samples contained 0.88, 0.83, and 0.79 g. urea respectively. As a result of the marked decrease in urea content of the bran when the wheat was washed, without the urea content of the interior portions of the kernel being materially influenced, the flour contained 52.3% of the total urea present.

Discussion

The qualitative observation that solutions containing orthophosphate-P^{32} and urea-C^{14} penetrated the wheat kernel, especially around the germ, could be anticipated from studies which have appeared in the literature on the entry of water into the kernel. These researches have recently been reviewed by Hinton (6).

It has been shown that water enters relatively quickly around the germ (5,9) through a capillary system which extends into the germ at the point of attachment to the ear (3,4). Subsequently, the water taken up by the pericarp becomes distributed throughout the kernel during the tempering period. Although there is an inward movement of water through the whole surface of the seed coats, a barrier of low permeability exists (5,7,9). This barrier has been thought to be the hyaline layer, but Hinton's recent paper (6) has reported measurements by a

direct method of the rate at which water can pass through different kernel tissues. Hinton found that the testa was the bran layer which offered the greatest resistance to the entry of water. There was no relationship between permeability of the testa and kernel size, grade of wheat, thickness of seed coat, or exposure of the wheat to adverse weathering conditions. In the present study it was not possible to identify the particular seed coat which acted as a partial barrier to orthophosphate and urea.

The quantitative studies on the distribution of inorganic phosphate and urea in the various mill streams from the Buhler mill clearly show that while bran presents a considerable barrier to the entry of these two major urinary constituents, they can penetrate to the endosperm at different rates and contaminate the flour. The degree of penetration observed in these experiments must not, however, be interpreted as a measure of the extent to which the flour might be contaminated in commercial milling practice. The high level of contamination employed, the uniformity with which the solutions of orthophosphate and urea were distributed among the kernels, and the short experimental milling system would all favor greater contamination of the flour than would be expected in commercial operation. Laakso *et al.* (8) found that rat urine had such a high spreading power that one rat excreting an estimated 8.0 ml. of urine per day would contaminate 10,000 kernels of wheat (approximately 350 g.) when allowed to roam over the surface of the grain with a supply of distilled water available. This is equivalent to about 46 ml. of urine per 2000 g. of wheat, in contrast with 100 ml. of solutions of urea and of orthophosphate corresponding in concentration to the levels of these substances in rat urine which were used in the experiments reported in this paper. In commercial practice only the wheat within a few inches of the surface of the grain in storage would be contaminated, and during handling these kernels would become mixed with uncontaminated grain. Moreover, the extent to which urine would penetrate those kernels which were contaminated would be somewhat restricted by the limited amount of water available. On the other hand, the subsequent tempering process will favor the further penetration of any urinary constituents which have entered the seed coats.

The fact that urea and orthophosphate penetrated the seed coats and that dry-scouring or washing the wheat had little influence on the amounts of these constituents in the flour fractions emphasizes the importance of control measures to prevent rodents from gaining access to wheat.

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A STUDY OF THE RELATION BETWEEN TIME, TEMPERATURE, MOISTURE CONTENT, AND LOAF VOLUME BY THE BROMATE FORMULA IN THE HEAT TREATMENT OF WHEAT AND FLOUR[1]

H. A. Becker and H. R. Sallans[2]

ABSTRACT

Study of the factors time, temperature, moisture content, and loaf volume in heat-treated wheat and flour indicates that denaturation of the gluten is a first-order reaction. The rate constant, derived by expressing loaf volume for the treated sample as a fraction of the control, shows a temperature dependency which follows the Arrhenius equation. Computation of the energy of activation for the reaction gave an average value (virtually constant) of 64.6 kcal. per mol. over the range 8–20% moisture for wheat. Correlation of these data shows that the wheat temperature required for a 3% decrease in loaf volume in 1 hour varies from 200°F. at 8% to 135°F. at 20% moisture content.

The thermodynamic magnitude involved in activation ΔF^*, ΔH^*, and ΔS^* are evaluated and indicate that water acts as a catalyst in the denaturation by increasing the entropy of activation, by decreasing the energy of activation, or both.

The effect of elevated temperatures on wheat and its products has been the subject of considerable research. Attention has been focused on two important problems: first, the properties of heat-treated flours, especially with regard to modification or improvement of baking properties; and second, the conditions under which wheat can be safely dried without injury to its baking properties. More recently, Pence *et al.* (5) studied the effect of moisture content, temperature, pH, and salt concentration on the rate of denaturation of gluten by heat, using solubility and baking properties as criteria of denaturation. However, no quantitative analysis of the relation between the controlling variables and the baking properties of heat-treated wheat and flour has yet been reported.

Subjection of wheat or its products to excessive heat treatment results in damage to the quality of the gluten. It is generally agreed that this damage is caused by thermal denaturation of the constituent proteins (2, 3, 5). To obtain a practical analysis of the relations involved, it is necessary that the baking test used must clearly reflect the extent to which the proteins have been denatured.

It has been shown by Geddes (2) that heat damage in wheat products is most clearly revealed by the baking test, using the stand-

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ard formula with the addition of 0.001% potassium bromate. Geddes (3) has also shown that a high positive correlation exists between the loaf volume determined by the bromate formula and the colloidal properties of the flour proteins, such as the viscosity of leached, acidulated flour suspensions and the peptization of the proteins by 1 *N* magnesium sulfate. It may, therefore, be concluded that loaf volume is a sensitive index of physico-chemical changes in the proteins and may be used to measure heat damage (5).

Larmour (4) has shown that loaf volume by the bromate formula is closely correlated with protein content of wheat. The relation was found to be linear and the regression equation

$$\text{Loaf volume} = 50.9\,(\text{protein content}) + 24$$

was found to apply over the range 7.0–16% protein. Thus loaf volume is directly proportional to the undenatured protein of sound wheat.

If the following assumptions are made about the mechanism of the thermal denaturation of the gluten proteins, an analysis can be made of the relations among time, temperature, moisture content, and loaf volume by the bromate formula. The assumptions which form the working hypothesis are:

(i) The thermal denaturation of gluten proteins is a first-order, monomolecular process;

(ii) the gluten proteins affecting loaf volume are all denatured at the same rate;

(iii) a protein molecule is either completely native or completely denatured; and

(iv) loaf volume is directly proportional to the concentration of undenatured protein.

The thermal denaturation of the proteins should follow the kinetic equation of a first-order reaction, and the concentrations of reactant and product (native and denatured protein) can be expressed in terms of the loaf volume. Pence *et al.* (5) made a similar assumption in studying the denaturation of gluten. The rate equation is therefore given by

$$k_r = (2.303/t)\ \log\ 1/V,$$

where k_r is the rate constant, sec.$^{-1}$;

t is the duration of the heat treatment, sec.;

V is the ratio between the final and initial loaf volume determined by the bromate formula; and

1/V is the ratio between the initial and final concentrations of native protein.

If the denaturation shows the characteristics of a first-order reaction, then log 1/V should be a linear function of the time of treatment.

Quite extensive data on heat-treated flours are available in the literature (2), but data on wheat are limited. It was decided to obtain additional data on wheat and to try to correlate the effects of time, temperature, and moisture with heat damage for both flour and wheat.

Materials and Methods

The Thatcher wheat selected for study was of No. 2, second-generation, registered seed grade grown in 1953. Flour milled from this wheat to 70% extraction showed a protein content of 15% (14% moisture basis) and gave loaf volumes of 905 cc. by both the standard and bromate formulas. Since both protein content and loaf volume were high, the wheat was considered to be well suited for a study of damage by heat.

At the time of tempering, the wheat showed a moisture content of 8.0%.[3] One lot of this wheat was retained without tempering, while three other lots of 15 lb. each were tempered to moisture contents of 10, 12, and 16% respectively. The wheat was then stored in 2½-gal. containers and allowed to condition for 1 week at room temperature before tests were begun.

The apparatus used was similar to that described by Geddes (2). The sample cylinder was constructed from a section of aluminum irrigation pipe 4 in. in diameter by 15 in. long. The ends were closed with disks of ¼-in. aluminum; one was welded, the other flanged and bolted. Shafts of ½-in. aluminum were welded to the disks. Four baffles made from ½-in. strips of 1/16-in. aluminum were welded longitudinally at 90° intervals around the inner periphery of the cylinder to promote mixing. The cylinder was mounted horizontally in an angle-iron frame and driven by a ¼-H.P. motor coupled to a variable speed unit. In operation the apparatus was placed in a constant-temperature oil bath with the cylinder completely submerged in the oil.

In making a run about 1200 g. of wheat were placed in the apparatus. The cylinder was immersed in the oil bath and rotation immediately begun. The speed used was just below that which caused the wheat to cling to the periphery of the cylinder. The initial flow of heat to the apparatus and its contents was very rapid, and it was found beneficial to overheat the oil bath by 4°–5°F. before beginning operation. This prevented the bath from dropping below the controlled temperature in the first few minutes of a run. It was found that the temperature of the wheat was within 1°–3°F. of the controlled temperature within 15 minutes after the start of the operation. All runs were of 2 or 3 hours' duration.

[3] All moisture contents in this paper are expressed on wet basis.

On completion of a run the cylinder was immediately transferred to a cold-water bath and rotated to ensure rapid cooling of the wheat. Moisture determinations were made before and after heat treatment, and only in a few cases was there a significant difference.

After treatment the samples were air-dried and stored at 11°C. until milling and baking could be completed. They were then given a preliminary tempering to 13% moisture and finally raised to the normal level of 15% immediately prior to milling. Straight-run flours were then produced on an Allis Chalmers experimental flour mill. The flours were baked using a bromate concentration of 1 p.p.m.

It will be noted in Table I that loaf volumes for the controls show a range of 905 to 970 cc. This probably reflects changes in the samples caused by tempering and may be due either to changes in the physical structure of the grain or to enzymatic action at the higher moisture levels. These effects are not uncommon and previous workers have noted loaf volume increases on drying. It was deemed advisable to minimize these effects in the present study by making treatment of the

TABLE I

SUMMARY OF HEAT TREATMENTS ON WHEAT

Moisture (Wet Basis)	Temperature		Time	Loaf Volume[a]	V	k_T, $sec.^{-1} \times 10^4$
%	°F.	°C.	hours	cc.		
16.1	Control		0	970	1.000	....
	145	63	3	895	0.922	0.075
	150	66	3	690	0.711	0.316
	160	71	2	465	0.480	0.980
	170	77	2	255	0.263	1.81
	175	80	2	290	0.299	1.68
12.1	Control		0	950	1.000	....
	165	74	3	802	0.845	....
	170	77	3	707	0.745	0.275
	180	82	2	467	0.492	0.980
	185	85	2	300	0.316	1.61
	190	88	2	200	0.295	1.69
10.3	Control		0	930	1.000	....
	170	77	3	890	0.937	0.041
	175	80	3	890	0.937	0.041
	180	82	2	860	0.905	0.112
	190	88	2	545	0.574	0.740
	195	91	2	410	0.432	1.14
8.0	Control		0	905	1.000	....
	190	88	3	890	0.984	....
	195	91	3	850	0.940	0.060
	205	96	2	820	0.906	0.138
	210	99	2	775	0.856	0.217
	215	102	2	605	0.668	0.560

[a] Basic formula + 0.001% bromate.

controls parallel as closely as possible the treatment of samples under investigation.

Results and Discussion

Geddes (2) has reported a series of data carried out on flour at 13.9% moisture. Figure 1 shows a plot of 2.303 log 1/V calculated from these data as a function of the time of treatment at different temperatures. The relation is linear and this proves that the first-order equation is applicable to this case.

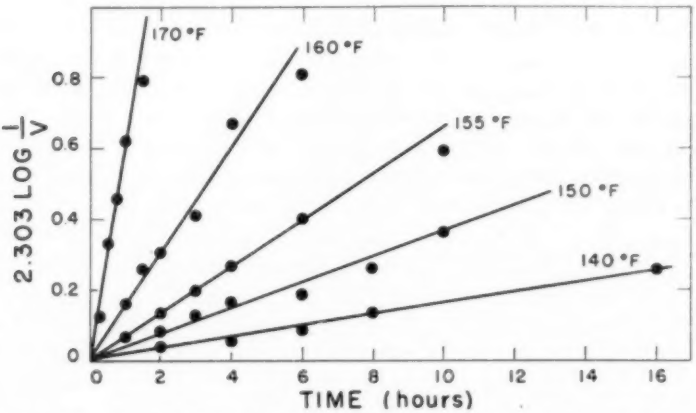

Fig. 1. The denaturation of gluten in flour at 13.9% moisture at various temperatures as measured by the loaf volume by the bromate formula. Data from Geddes (2).

The results for wheat are summarized in Table I, which also gives the calculated values of the first-order rate constant, k_r. It should be noted that the value of k_r can be considered accurate only when the ratio V is less than 0.95 and the loaf volume is greater than about 300 cc. Loaf volume by the bromate formula reaches a minimum between 200 and 250 cc. A certain minimum value must exist because of the volume occupied by the ingredients of the loaf, and as this minimum is approached the relation between damage and loaf volume fails. Larmour's results (4) indicate that a volume of 300 cc. corresponds to a native protein content of about 5.5%, and it can be assumed that at about this point the normal relation between loaf volume and the content of native protein breaks down. This serves to emphasize the importance of using a high-protein wheat when studying heat damage.

The first-order rate constant should show an Arrhenius-type temperature dependency. This may be expressed by the equation:

$$\log k_r - \log A = (-E/2.303RT),$$

where E is the energy of activation, cal. per mol.;

A is a constant, sec.$^{-1}$

T is the absolute temperature, °K; and

R is the gas constant, 1.987 cal. per mol. °K.

Figure 2 shows log k_r computed from data on wheat as a function of 1/T for various moisture levels. The relation appears to be essentially linear up to 20% moisture content. Since there were not sufficient data to justify differentiation between the curves, an average value for the energy of activation, E, was calculated, using all the data except those for a moisture content of 21.6%, and the curves in Fig. 2 have been drawn with a constant slope corresponding to this value of E. This procedure may not be entirely valid theoretically but it assists in the practical interpretation of the results. The calculated average value of E was 64.6 kcal. per mol. This is in the range reported as typical of protein denaturations (6).

If it is assumed that the energy of activation is constant, then the constant, A, in the Arrhenius equation is obviously a function of moisture content. Figure 3 shows the values of log A calculated from the curves in Fig. 2 as a function of the moisture content. Specification

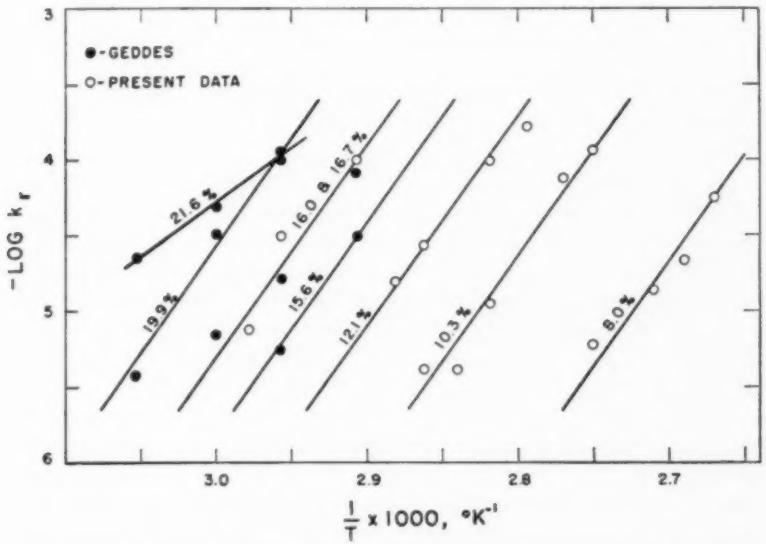

Fig. 2. Relation between temperature and the rate of denaturation of gluten in wheat at various moistures as measured by the loaf volume by the bromate formula. Curves for 8.0 - 19.9% moisture drawn with a constant slope corresponding to an energy of activation of 64.6 kcal. per mol. The energy of activation at 21.6% moisture is 32.7 kcal. per mol.

of both E and A allows calculation of the first-order rate constant at any given temperature. Hence, when combined with the value of the energy of activation E = 64.6 kcal. per mol., Fig. 3 reflects the general

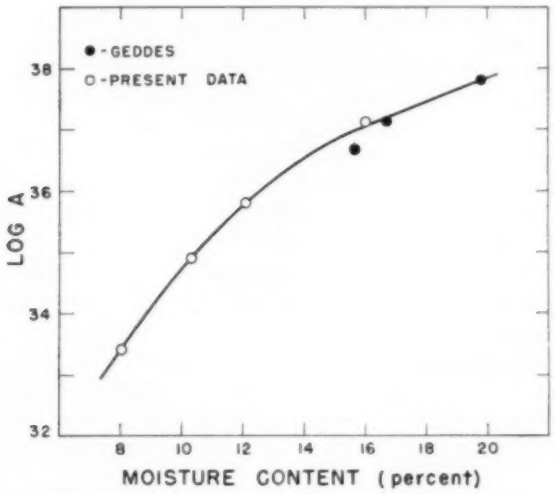

Fig. 3. The constant in the equation log k_T — log A = −64,600/2.303RT as a function of the moisture content of wheat in the range of moisture 8 - 20%. Computed from Fig. 2, in which the averaged slope of the curves for different moistures corresponds to an energy of activation of 64.6 kcal. per mol.

relationship between loaf volume by the bromate formula, time, temperature, and moisture content for wheat in the range of moistures 8–20%.

At any given temperature Fig. 3 gives essentially the relation between the rate constant and the moisture content of wheat. The figure indicates that in the range of moistures shown the rate constant is strongly dependent on moisture content. Increasing the moisture from 8% to 20% increases the rate constant by a factor of more than 10^4. The rate of increase is greatest at the lower moistures: between 8% and 13% the constant increases by a factor of 600, but between 15% and 20% it increases by a factor of 10.

Figure 4 shows an interpretation of Fig. 3 in terms relevant to the practice of wheat drying. The figure shows the temperature necessary for a decrease in loaf volume of 3% in 1 hour as a function of moisture content. Since the retention time in the drying section of a hot-air drier is normally less than 1 hour, this figure gives the approximate maximum grain temperatures at which wheat can be dried without significant damage to its baking properties.

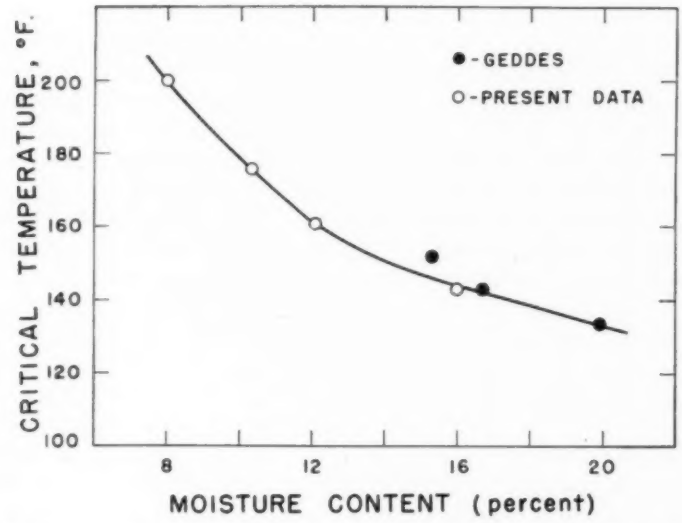

Fig. 4. A practical interpretation of Fig. 3 in terms relevant to the practice of the drying of wheat, showing the temperature required for a decrease in loaf volume by the bromate formula of 3% in 1 hour.

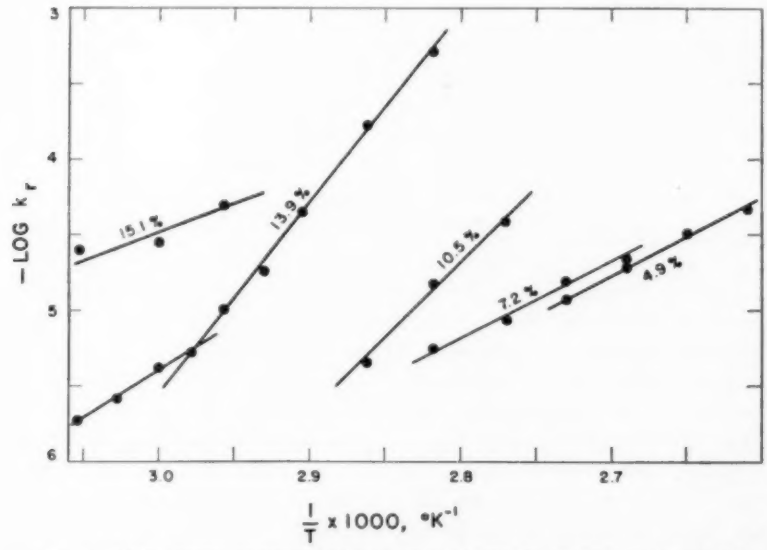

Fig. 5. Relation between temperature and the rate of denaturation of gluten in flour at various moistures as measured by the loaf volume by the bromate formula. Data from Geddes (2).

Figure 5 shows the rate constants calculated from data on flour (2) as a function of the reciprocal of the absolute temperature. The data for 13.9% moisture are average values, k_r corresponding to the slopes of the curves in Fig. 1. It is evident that the relation plotted is essentially linear. However, unlike that for wheat, the proportionality between log k_r and 1/T for flour clearly depends on the moisture content. The data for 13.9% moisture appear to indicate that it also depends on the temperature, since there is an abrupt change in the slope of the curve at about 63°C. (1/T = 2.98). However, it should be noted that the rate constants shown for this moisture are average values. Table II shows the actual calculated values of the first-order rate constant for different times of treatment at temperatures of 57° and 68°C.; above 63°C. the rate constant is independent of time, whereas below 63°C. it decreases with increasing time of treatment. This suggests that below 63°C. thermal denaturation is of secondary importance to some type of reaction that is inhibited by higher temperatures. It may be assumed that this reaction is gradually stopped by heat inactivation of proteolytic enzymes.

TABLE II

HEAT-TREATMENT OF FLOUR AT 13.9% MOISTURE: BEHAVIOR OF THE FIRST-ORDER RATE CONSTANT ABOVE AND BELOW 63°C.[a]

Temperature = 57°C.		Temperature = 68°C.	
Time	k_r, sec.$^{-1}$ × 10^4	Time	k_r, sec.$^{-1}$ × 10^4
hours		*hours*	
2	0.071	1	0.192
4	0.045	2	0.182
8	0.029	3	0.183
16	0.027	4	0.186
24	0.022	6	0.187

[a] Computed from data of Geddes (2).

Energy Relations. The Eyring formulation of rate theory as interpreted by Stearn (6) for protein denaturation may be applied to the data. Eyring's theory emphasizes the free energy of activation rather than the energy of activation. The reaction may be symbolized by

$$N \rightleftharpoons X^* \rightleftharpoons D,$$

where N denotes native protein, X^* denotes the intermediate activated complex, and D denotes denatured protein. If K^* represents the equilibrium constant for the formation of activated molecules, then K^* is related to the first-order rate constant by

$$k_r = \kappa\ (kT/h)\ K^*$$

where κ is a transition coefficient assumed to equal unity,

k is the Boltzmann constant, and

h is Planck's constant.

The equilibrium constant K* is related to the thermodynamic magnitudes involved in activation by

$$\Delta F^* = -2.303\ RT\ \log\ K^*$$
$$= \Delta H^* - T\Delta S^*$$

where ΔF^*, ΔH^*, and ΔS^* are the standard changes in free energy, heat content, and entropy, respectively, for the formation of activated complex from reactant molecules. ΔH^* is related to the energy of activation E by

$$\Delta H^* = E - RT$$

Thus

$$k_r = A \exp - E/RT$$
$$= (kT/h) \exp - \Delta F^*/RT$$

The foregoing equations facilitate the calculation of ΔF^*, ΔH^*, and ΔS^*.

Table III gives the experimental values of E and log A, and the calculated values at 50°C. of ΔF^*, ΔH^*, and ΔS^* from data on wheat, on flour (2), and on gluten (5). It may be seen that both E and ΔF^* are in the ranges common for protein denaturation, and the formation of activated complex is accompanied in most cases by a large increase in entropy.

Following the interpretation of Stearn (6), it appears that one of the steps in activation is hydrolytic cleavage of a cystine bridge, involving 20 kcal. per mol. but resulting in negligible entropy increase. The other steps involve the breaking of a number of weaker bonds with an entropy increase of about 12 e.u. per bond. Stearn assumes the strength of these bonds as 4 kcal. per mol., but a value of 5 kcal. per mol. gives better agreement with his data when the number of bonds broken is less than 15. Hence it may be supposed that the number of weak bonds broken per molecule in activation is

$$(\Delta H^* - 20{,}000)/5{,}000 \text{ or } \Delta S^*/12$$

and in addition one cystine bridge is broken with zero increase in entropy.

The last two columns in Table III show the number of weak bonds broken per molecule as given by the two methods of calculation. In all cases where two or more weak bonds appear to have been broken, the two estimates do not differ by more than one bond. The data for wheat in the range of moistures 8.0–19.9% indicate the rupture of nine weak bonds in addition to the cystine bridge. With flour at 13.9%

TABLE III

THERMAL DENATURATION OF WHEAT PROTEINS AT 50°C.

Moisture	E	log A	ΔF^*	ΔH^*	ΔS^*	$\frac{\Delta H^* - 20}{5}$	$\frac{\Delta S^*}{12}$
%	*k cal/mol*		*k cal/mol*	*k cal/mol*	*k cal/mol*		
WHEAT[a]							
8.0	64.6	33.5	34.1	64.0	92.6	8.8	7.7
10.3	64.6	34.9	32.0	64.0	99.1	8.8	8.3
12.1	64.6	35.8	30.7	64.0	103.2	8.8	8.6
15.6[b]	64.6	36.6	29.5	64.0	106.8	8.8	8.9
16.0, 16.7[b]	64.6	37.1	28.7	64.0	109.1	8.8	9.1
19.9[b]	64.6	37.8	27.8	64.0	112.2	8.8	9.3
21.6[b]	32.7	17.1	26.4	32.1	17.6	2.4	1.5
FLOUR[b]							
4.9	23.6	9.15	29.7	23.0	−18.8	0.6	0?
7.3	23.6	9.25	29.5	23.0	−18.3	0.6	0?
10.5	45.3	23.0	30.3	44.7	44.6	4.9	3.7
13.9	57.9	32.4	29.0	57.3	87.6	7.5	7.3
GLUTEN[c]							
64–67	34.6	18.3	26.6	34.0	22.9	2.8	1.9
64–67[d]	43.8	23.5	27.8	43.2	47.7	4.6	4.0

[a] E assumed constant at moistures of 8.0–19.9%.
[b] Calculated from data of Geddes (2).
[c] Calculated from data of Pence *et al.* (5).
[d] Determined by solubility method.

moisture, seven or eight weak bonds appear to be involved, but at 10.5% moisture the number apparently decreases to four or five, and at 7.2% and 4.9% moisture it appears that the activation may involve only the rupture of a cystine bridge. However, the negative entropy change of about −19 e.u. in this last case is not completely consistent with the mechanism pictured. With wheat at 21.6% moisture, two weak bonds appear to be broken. The baking test method of measuring denaturation in gluten indicates the rupture of two weak bonds, but the solubility method indicates twice as many. However, Pence *et al.* (5) have noted that the solubility method affects only about 75% of the gluten and it is the less soluble glutenin remainder which is most susceptible to denaturation.

Effect of Moisture. It has been shown recently that the coefficient for the diffusion of moisture out of wheat decreases by a factor of 10 to 25 as the moisture content falls below a level of 8.5–9.3% (1). Consequently the marked change in the process of denaturation shown by flour as the moisture falls below this level is not surprising. It is prob-

able that this change is also shown by wheat, since the data for 8.0% moisture in Fig. 3 are fitted better by an energy of activation of 42 kcal. per mol. than by the average value of 64.6 kcal. per mol.

The relation among the thermodynamic magnitudes involved suggests that activation may be initiated by cleavage of a cystine bridge. It may be supposed that this unlocks the folded structure of the protein molecule. Whether or not this is followed by rupture of weaker bonds holding the molecule in its folded configuration depends upon the availability of water molecules. Thus at low moistures only the initiating reaction occurs, whereas at higher moisture it is followed by the rupture of as many as nine of the weaker (probably hydrogen) bonds. Whatever the true mechanism may be, all results indicate that water can be looked upon as a catalyst which in the range of temperatures 50°–90°C. increases the rate of denaturation by increasing the entropy of activation, by decreasing the energy of activation, or both.

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THE HEATING CHARACTERISTICS OF AN AMYLOGRAPH[1]

STEPHEN J. LOSKA, JR.,[2] AND JAMES A. DAY[2]

ABSTRACT

Thermocouple probings during amylograph tests indicated that the heating of the suspension in the cup of this machine is uniform from top to bottom and from the outer radius to the axis of rotation. The exceptions appear to be in the range of 90° to 95°C. A soft-wheat flour suspension having the amylogram peak in this range exhibited larger temperature gradients than a malted hard-wheat flour suspension. The viscosity of the hard-wheat suspension was considerably less in this temperature range, the peak of the curve having occurred at a lower temperature.

The simplicity of design of the Brabender Amylograph, the position of the thermoregulator, the use of a spiral wire for the contact mover, and the position of the heater coil all appear to be inadequate to provide uniform heating of the flour-water suspension. Johnson (1), in a report for the Amylograph Standardization Committee, noted that the data suggest that some variation exists in the various thermoregulators used. In the tests several thermometers and operators were involved. The report qualifies the interpretation by acknowledging that the inability to correctly place an auxiliary thermometer and inadequate stirring might be the cause of some of the variation.

The purpose of this investigation was to determine the temperature uniformity of flour-water suspensions during the heating cycle and to determine causes of any variations that may be noted.

Materials and Methods

The amylograph used for this test series was Number 303, Type AC7, purchased in 1953. Trials were made using two flours at different concentrations in order to cover the typical conditions under which the amylograph is used. Suspensions of 65 g. of a soft-wheat patent flour and 100 g. of a malted hard-wheat patent flour in 460 ml. of distilled water were used. Flour weights were corrected to a 14% moisture basis. The amylograph values of the soft- and hard-wheat flours were 780 A.U. and 500 A.U. respectively.

A small mass iron-constantan thermocouple was prepared and inserted into a glass tube, and the lead wires were sealed off with a fiber plug above the exposed thermocouple junction. The couple and a Brown Electronik recorder were calibrated separately and in combina-

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tion. The net calibration curve was used to correct recorder values. All recorder temperatures indicated in the figures have been corrected by the combined couple-recorder correction curve. The cooling coil and the evaporation cover of the amylograph were removed during the tests, which is standard procedure in this laboratory. An A. Schuetz fractional-degree thermometer was used as the reference. The thermocouple was placed in the bowl at the radius of the thermoregulator and at the axis of bowl rotation. Repeated runs were made with each sample until several points in the bowl had been probed.

The amylograph curve was recorded for all tests where the position of the thermocouple would allow. When the couple was placed on the axis of cup rotation the spindle was secured in position and the spring and upper shaft assembly lifted out of operation.

The indicating and controlling accuracy of the amylograph regulator-thermometer was checked against the standard thermometer by making and breaking contact in water of known temperature.

Results

Figure 1 shows the indicating-controlling calibration of the thermoregulator at points every 5°C. over the useful temperature range. The difference between points of make and break was within 0.1°C. which is the limit of accurate scale readability.

The regulator temperature agreed exactly with that shown by the standard thermometer over the entire heating range of 25° to 90°C.

Figure 2 shows the results of the regulator driving rate tests. For these trials it was not necessary to have the amylograph operating under test conditions. The heater coil was disconnected and the regulator then tested. The dashed reference line in Fig. 2 is the theoretical indicator position plotted against time. Between 7 and 10 minutes the rate was faster than the 1.5°C. per minute design of the thermoregulator. This resulted in a slight lateral shift in the time-temperature curve.

A test system would be slightly hotter than the time would indicate, though the regulator is controlling properly. This is probably because of nonuniformity in the spiral pitch of the wire contact mover.

Figure 3 shows the time *vs.* temperature curve during a normal heating cycle with the water in the cup. The position of the thermocouple is indicated by a small cross on two views, top and side, of the bowl and thermoregulator. The cross placed near the edge of the circle indicates the thermocouple at a position on the same radius as the center of thermoregulator bulb. A slight temperature gradient was evident at the upper temperature limits. The curve for the thermo-

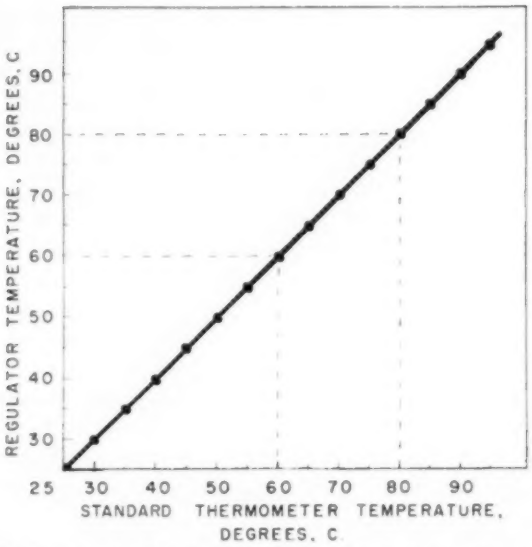

Fig. 1. Calibration curve for indicating accuracy of amylograph thermoregulator measured against the standard thermometer.

couple on the same radius as the thermoregulator followed the theoretical line better than the couple at the center of rotation. This is

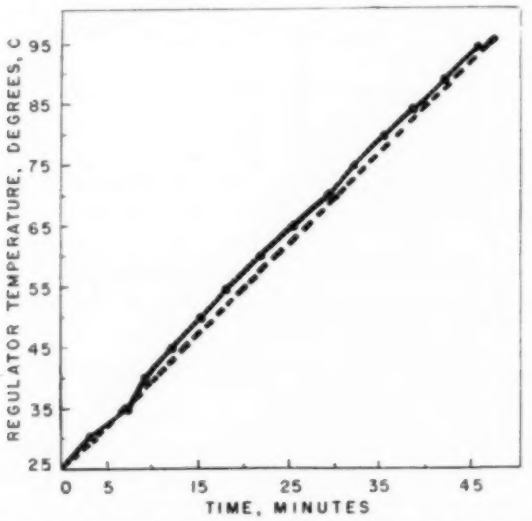

Fig. 2. Calibration of amylograph thermoregulator drive expressed as indicated regulator temperature versus time. The dotted line represents the theoretical 1.5° per minute driving rate.

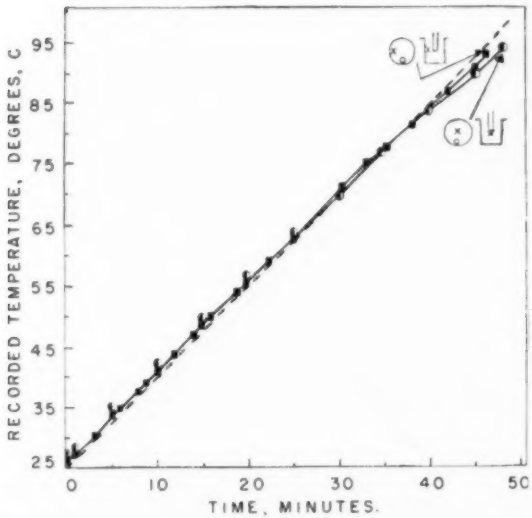

Fig. 3. The time-temperature curves of water during the normal amylograph heating cycle. The position of the thermocouple in this and succeeding figures is indicated by a small cross in the legend showing the top and side views of the thermoregulator and bowl. The dashed line represents the theoretical temperature rise of 1.5°C. per minute. The solid lines represent the temperatures recorded at the different thermocouple positions.

in line with regulator design practice. When the controller is placed near the heat source the material beyond the regulator (toward the center) should be at a lower temperature. With agitation in the cup this is evident only at the high temperatures.

Figure 4 shows the time-temperature curves at three positions of the thermocouple on the same radius as the thermoregulator bulb during amylograph tests using soft-wheat flour (65-g. basis). The positions of the thermocouple were at the bottom, middle, and top of the suspension as indicated by the legends illustrating the top and side views of the bowl and thermoregulator. The time at which maximum viscosity occurred is marked on this and the following figures by a cross placed on the time axis. Measurements near the surface were difficult in the range of maximum viscosity because of a tendency of the slurry to form a cavity exposing the couple.

Figure 5 shows the time-temperature curves at two positions, bottom and middle, along the axis of rotation during tests on the same flour.

Temperature gradients were evident again. Except for points at the very bottom outside radius and along the axis of rotation, the gradients are not serious. The greatest deviations at high temperatures are

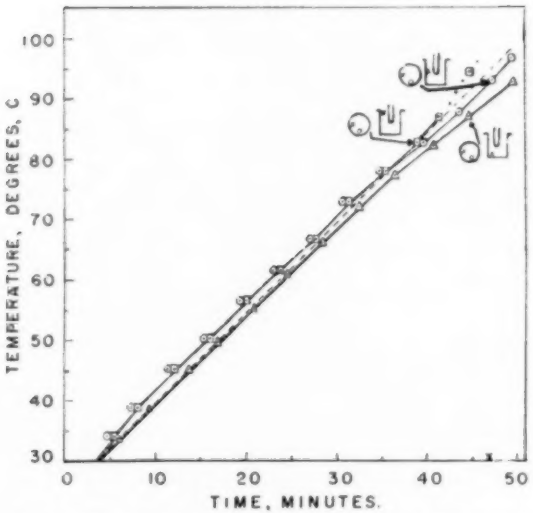

Fig. 4. The time-temperature curves of the thermocouple probings at the bottom, middle, and top of the bowl on the same radius as the thermoregulator during the amylograph tests of a soft-wheat flour. The time of the amylogram peak is designated by a cross on the time axis.

along the axis of rotation, and accompanying viscosity changes would hardly be evident because of the absence of spindle rods near the axis.

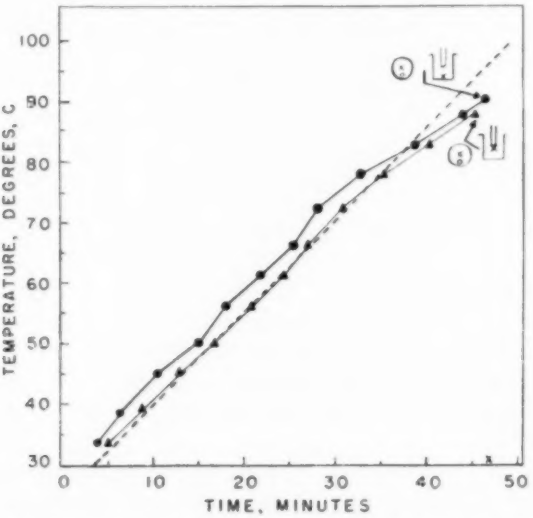

Fig. 5. The time-temperature curves of thermocouple probings at the bottom and middle of the bowl along the axis of rotation during the amylograph heating cycle of the soft-wheat flour used in Fig. 4.

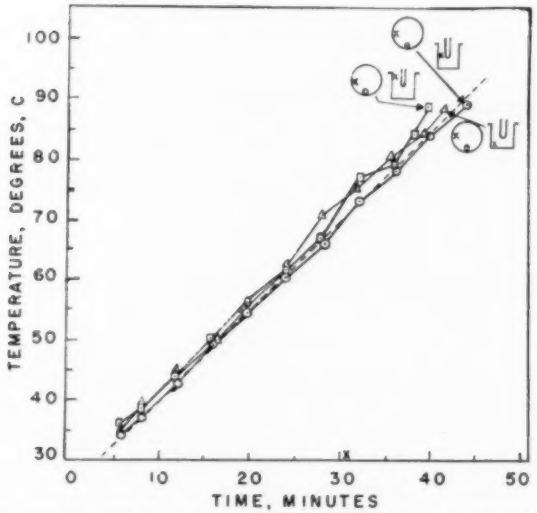

Fig. 6. The time-temperature curves of thermocouple probings at the bottom, middle, and top of bowl on the same radius as the thermoregulator during amylograph tests of a malted hard-wheat flour. The time of the amylogram peak is designated by a cross on the time axis.

Figures 6 and 7 show the time-temperature curves of thermocouple positions during an amylograph test of a malted hard-wheat flour

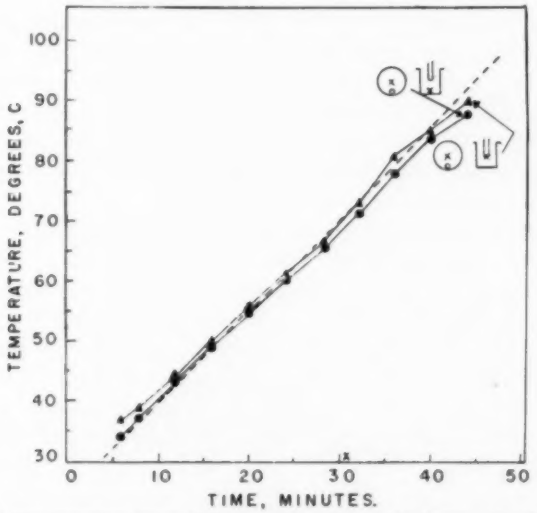

Fig. 7. The time-temperature curves of thermocouple probings at the bottom and middle of the bowl along the axis of rotation during the amylograph heating cycle of the malted hard-wheat flour used in Fig. 6.

(100-g. basis). Measurements near the surface were difficult, again because of the couple exposure.

The deviations at high temperatures were less than the deviations with the cake flour slurry. At the high temperatures where a temperature gradient exists the curve is well beyond the peak, indicated by the cross on the time axis, and the viscosity is considerably lower than at the peak. Gradients at high temperatures would not affect the amylogram peak of a malted hard-wheat flour.

A slight upward bend in the curve is evident at the point of maximum viscosity. No interpretation can be given, but it was evident in all five probings.

Discussion

The thermocouple probings during the amylograph test indicate that the heating of the slurry in the cup of this machine is uniform from bottom to top and from the outer radius to the axis of rotation. The exception appears to be in the range of 90° to 95°C., particularly with the flour having maximum viscosity in this range. The temperature recording showed only a slight tendency to overshoot the point when regulator contact was made and the heat turned off. The dissipation of the latent heat in the coil, air, and bowl during the heat-off cycle raises the temperature of the slurry at a rate very nearly the same as the contact movement of 1.5°C. per minute. The heat cycle operates to drive the mercury of the regulator up to the contact.

The data indicate that the spindle and bowl pins supply enough agitation to prevent localized heating. Compared to the size of the mercury bulb, the thermocouple makes a measurement at a small point. The small deviations from the theoretical curve measured by a thermocouple near the bulb may not be important, since the temperature of the thermoregulator is in effect a mean temperature at the surface of the mercury bulb.

Other than the routine check for proper machine operation, two checks are advisable if the machine is to be used for accurate work: (1) The accuracy of the thermometer should be checked against a calibrated one. This will reveal the most common fault of a shortened contact wire due to returning the contact to the starting position too fast. (2) The uniformity of the regulator spiral should be tested by time runs. The lost motion or slack in the gear train and spiral contact mover should be removed after the contact has been returned to the starting point.

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RELATIONS BETWEEN WHEAT PROTEIN CONTENT, LOAF VOLUME, EXPANSION VOLUME, AND SEDIMENTATION VALUE

II. Samples Delineated by Particle Size[1]

R. H. Harris and L. D. Sibbitt[2]

ABSTRACT

Protein content and loaf volume tended to increase inversely with particle size in two series of composite flours milled from hard red spring and Mexican wheats respectively until mesh No. 400 was reached. At this point a marked decrease in both properties occurred. Expansion volume and sedimentation value tended to increase with protein content and loaf volume. No relation existed between particle size and the other flour properties measured unless the results with sieve 400 were disregarded, when very significant correlations were obtained.

Harris and Sibbitt (2) showed that results obtained with the expansion volume method of Miller, Edgar, and Whiteside (3) and the sedimentation test of Zeleny (5) provide useful supplementary information to that furnished by protein determinations with hybrid wheats of very diverse quality. But for wheats of generally superior quality, protein content, expansion volume, and sedimentation value had little relation to loaf volume. The authors concluded that little advantage accrued from use of either expansion volume or sedimentation value tests in preliminary screening of nursery samples for quality.

The present study was undertaken to ascertain whether expansion volume and sedimentation value were related to other flour properties in two series of flours which differed in particle size within the group (1). One series was from a composite flour milled from standard hard red spring wheat varieties; the other was from a composite flour milled from a series of wheats grown in Mexico. Thus the influence of wheat variety was removed. Separation and properties of the hard red spring wheat series have been described by Harris (1). The Mexican samples were similarly prepared from a composite of residues.

Methods used in the expansion volume, sedimentation value, and baking tests have been published (2, 3, 5) and will not be described.

Results

Table I shows comparative data obtained from the two series of flour differentiated by particle size within each group (1). Protein

[1] Received for publication October 31, 1955. Published with the approval of the Director, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

[2] Department of Cereal Technology, North Dakota Agricultural Experiment Station.

TABLE I

DATA OBTAINED FROM FLOURS OF DIFFERENT PARTICLE SIZES

Mesh Size	Flour Protein	Loaf Volume	Expansion Volume	Sedimentation Value	Specific Sedimentation Value
	%	*cc.*	*ml.*	*ml.*	
	Mexican hybrid composite				
Original flour	9.5	150	11.8	25.4	2.7
115	8.8	115	...[a]	19.1	2.2
150	9.6	135	13.4	25.1	2.6
200	10.4	140	16.6	30.6	2.9
250	11.1	145	16.2	34.4	3.1
270	11.7	160	14.2	37.7	3.2
400	7.6	115	10.4	...[b]	...[b]
	Hard red spring composite				
Original flour	13.2	210	18.2	47.6	3.6
115	13.1	160	20.6	31.1	2.4
150	13.8	180	21.6	39.0	2.8
200	14.8	185	23.6	59.4	4.0
250	15.9	210	23.8	65.6	4.1
270	16.8	240	23.6	69.2	4.1
400	11.6	115	8.6	53.0	4.6

[a] Insufficient flour for expansion test.
[b] Impossible to read sedimentation value.

content and loaf volume in both series tended to increase inversely with particle size until the smallest size, passing mesh No. 400, was reached, when there was marked decrease in both properties. This agrees with the results of Wichser, Shellenberger, and Pence (4) and Harris (1), and is evidence that this relationship exists generally in flours milled either commercially or experimentally. Expansion volume and sedimentation value tended to increase with protein content and loaf volume. However, calculation of correlation coefficients revealed no relation at the 5% point of significance between particle size and any of the flour properties measured unless the results with sieve size 400 were disregarded, when very significant coefficients (+0.90 or better) were obtained with the different flour properties assayed.

Generally the conclusions on the utility of expansion volume and sedimentation value determinations for predicting baking quality derived from the hybrid samples (2) are confirmed by the results from flours of different particle size originating from the same wheat. Actually they tend to weaken the belief that the two proposed methods of

assessing quality offer any advantage over determination of protein content alone.

Acknowledgment

The authors wish to acknowledge the courtesy of the Cereal Crops Division of the Department of Agriculture, Ottawa, Canada, for the loan of their expansion apparatus for use in this investigation.

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5. ZELENY, L. A simple sedimentation test for estimating the bread-baking and gluten qualities of wheat flour. Cereal Chem. **24:** 465–475 (1947).

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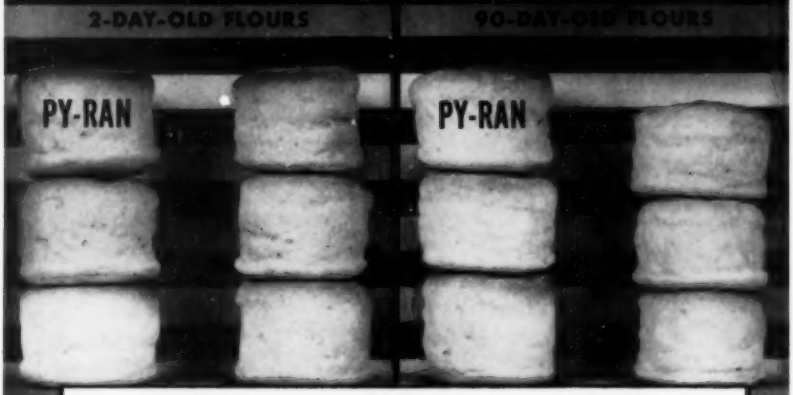

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The Commissioner said: "Under the Program, substantial progress has been made in improving handling and storage of wheat to prevent contamination. However, experience acquired during the past six months shows that the levels of judgment should be tightened if the program is to continue to be effective."

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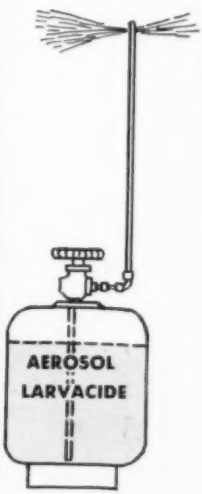

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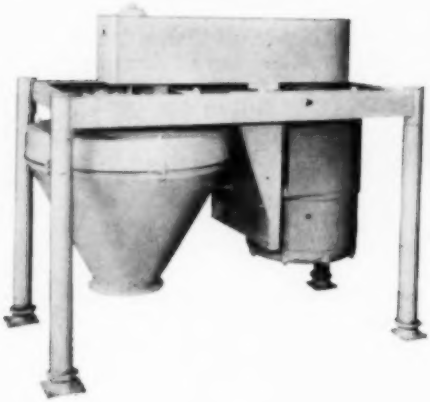

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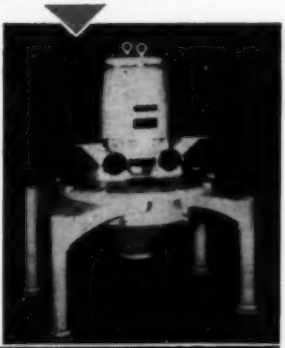

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